A GUIDE
TO THE IDENTIFICATION OF THE
GENERA
OF BACTERIA

BY **V. B. D. SKERMAN**

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1. bacteria
2. ~~genera~~
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A GUIDE
TO THE IDENTIFICATION OF THE
GENERA
OF BACTERIA

WITH METHODS AND DIGESTS OF
GENERIC CHARACTERISTICS

77

BASED ON DATA GIVEN IN THE SEVENTH EDITION OF BERGEY'S MANUAL
OF DETERMINATIVE BACTERIOLOGY AND ON ORIGINAL PAPERS

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THE WILLIAMS & WILKINS COMPANY
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TO MARGARET BREED
in appreciation of a silent service to microbiology

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CONTENTS

PREFACE v

INTRODUCTION vii

A Comprehensive Key to the Genera of *Bacteria* 1

Digest of Genera 48

Methods 143

A Guide to Study 207

INDEX 211

PREFACE

The Key to the Genera of Bacteria has been compiled with the aim of placing in the hands of research workers, teachers, and students, a volume in which general directives for the identification of bacteria are supported by a complete list of the techniques needed for the purpose. In addition, a Digest of data published for the various species in the seventh edition of *Bergey's Manual of Determinative Bacteriology* and in several original papers has been included. The publication of the Key and Methods in the one book should encourage a more general application of common procedures for the description of bacteria. The main purpose of the digests is to draw attention in a more definite way to the deficiencies in descriptions within the various genera in the hope that steps may be taken to rectify them. A Guide to Study has been provided to ease the burden associated with the assimilation of knowledge over this varied field of science.

The volume is intended as a supplement to the *Manual* itself, and it is hoped that its use will contribute materially to the development of future editions of the *Manual*.

I am indebted to the late Professor R. S. Breed, at whose invitation the present keys were written for inclusion in the seventh edition of *Bergey's Manual*, to the Board of Trustees of the *Manual* for permission to draw so freely on the contents of the *Manual*, and particularly to Dr. R. E. Buchanan for a very critical perusal of the manuscript.

I am also indebted to the numerous authors, organizations, and publishers who have so readily given their permission to reproduce the material which appears in the Methods and illustrations, and whose names appear in the references to the respective items.

In drawing up the Methods, I have made use of some which have been in use for a long period in this laboratory and for which the original references can not be traced. These methods bear no reference and acknowledgement is due to the original authors and publishers whoever they may be.

I should like to express my appreciation to the Carnegie Corporation of New York through whose financial assistance I was able to visit the late Professor Breed and many other people whose assistance to me in the compilation of the keys has proved invaluable; to Mrs. Anne Pope, Miss Barbara Carey, and Mr. Ian MacRae for the very great assistance which has made the publication of the volume possible; and to Miss Barbara Steele who typed the manuscript and whose unusual interest in the work has resulted in the elimination of many errors and omissions. I am also indebted to the Department of Photography, University of Queensland, for the preparation of line drawings and the development and printing of the photographic illustrations; and finally to those colleagues whose comments, acid and otherwise, have contributed to formulation of the Key.

V. B. D. Skerman
October, 1958

INTRODUCTION

The Key is essentially the same as that which appeared in the *Manual*. Certain additions have been made of genera which were omitted from the *Manual* or appeared in the literature after the manuscript had gone to press, and the material has been annotated more freely than in the previous key, although perhaps not as extensively as some may wish. A major alteration which has been made is the insertion at Section H**93** of the statement "Not as above . . . **94**." This became necessary following the application of this general comparative approach in our laboratory when it became obvious that numerous organisms which are not listed as doing so have the power of liquefying Loeffler's inspissated serum. The insertion provides a shunt for these organisms. A second major alteration is the inclusion of the keys for the Order *Myxobacterales* and the organisms in the Class *Microtatobiotes*. In both cases the keys are merely reconstructions of those provided in the *Manual* and are reproduced in this form with the permission of the authors and publishers.

The Key departs in several places from a simple dichotomy. Such departures occur where (*a*) there has been some doubt as to the validity of the insertion, and the use of other than a dichotomy permits subsequent removal without reconstruction and numbering of the key; (*b*) there has been a late insertion which would have necessitated a complete renumbering of the key; and (*c*) it appeared more convenient to depart from the dichotomy as in Section B.

Shunts have been used where there is a divided opinion on interpretation or a test is of limited usefulness. By this device, organisms may be separated into two groups by a dichotomy. Certain genera are then separated from one group on specified tests and the remaining organisms rechanneled into the other group for subsequent treatment. One such shunt has been used in the treatment of the multicellular organisms and another, in the separation of the cocci.

An alteration has been made of the definition of terms given as a footnote to Section A. The author has received some comments on the use of the term, trichome—all indicating that its use is undesirable. In this the author agrees but the term is still used in an attempt to define the different ways in which it has been employed in the *Manual*. Its elimination from subsequent editions of the *Manual* and from current literature is desirable.

The use of the term multicellular also warrants comment. Bisset regards organisms which, in their more stable rod form, are found to be composed of a small number of cells, as multicellular, *e.g.*, *Nocardia* and *Corynebacterium*. He does not regard an elongated rod similarly divided but into much longer cells as multicellular, *e.g.*, *Streptomyces*. This seems a little inconsistent and if applied to such genera as *Oscillatoria* or *Vitreoscilla* would give rise to the undesirable division of genera into multi- and unicellular species. In the Key those organisms which show a clear subdivision in the rods without resort to special staining methods are regarded as multicellular, *e.g.*, *Caryophanon*. Provision has been made, however, for those smaller cells such as *Nocardia* by means of a shunt in the Key which admits them as multicellular although special methods are needed to demonstrate this.

From cytological examination there is no suggestion that these cells are physiologically interdependent. That they may be so in some cases is evident from the great sensitivity of *Beggiatoa* to mechanical transfer in attempts to isolate it.

Page references following genera in the Key and in the Digest of Genera are in two forms. Those preceded by the letter *M* refer to the pages in the seventh edition of

Bergey's Manual of Derminative Bacteriology; the others refer to pages in this volume.

As with the previous Key, the alternative terminology given by Topley and Wilson (indicated by (T and W, 4th ed.)) is used in the Key wherever the correlation between the two terminologies could be clearly determined.

The Methods have been collected from many sources. Most of them have been tested in the author's laboratory. In most instances the original procedure has been followed, but, particularly in the case of synthetic media for autotrophic bacteria, a complete new series of alternative media based on a common synthetic base has been provided in addition to the original methods. The methods are not to be regarded as "standard methods." It is hoped, however, that general application of them in conjunction with the Key will lead to a greater uniformity in descriptions at the generic level. In the presentation of the methods a few procedures for isolation of cultures, which have a direct bearing on the operation of the Key, have been included.

A fundamental objection to the use of a common procedure is that there is no guarantee that it is, in fact, the one used by the original author, or, if so, that subsequent authors have used a similar procedure. The objection is valid, but it is not impossible, even now, to re-examine species on a common pattern and continue to use such a pattern in the future.

The present indiscriminate use of tests is leading only to chaos. The adoption of a more uniform approach should result in a much clearer picture of the present state of knowledge and should act as a stimulus to more productive research.

It is very desirable that some measure of international agreement be reached on the matter of methods.

The Digest of Genera has been prepared from an analysis of data given in the *Manual*. In many instances, particularly in the case of older German literature on the sulfur or iron bacteria and with more recently described genera, a search of the original literature has been made. The object of the Digest is to present the facts relating to the descriptions of species in a manner which accentuates the deficiencies With the exception of those organisms placed in the *Manual* in the Class *Microtatobiotes*, the digests are in two sections, namely, Differentiating Characters and Notes. The author has had no personal experience with the Class *Microtatobiotes* and has given under the heading of Differentiating Characters the generic description provided for each genus in the *Manual*. The differentiating characters given for the other genera are those derived by manipulation of the Key in reverse.

In the author's experience students automatically begin to trace keys backwards to determine the essential differentiating features. Because of numerous multiple insertions and the use of shunts in the Key, the practice is open to serious errors by those ignorant of the details of construction of the Key. For this reason the differentiating characters derived in this manner have been given. In the construction of the Key numerous assumptions had to be made to cope with lack of information. Where there has been any serious doubt the character has been treated as positive or negative with respect to the character. Where an assumption has appeared legitimate, such as the absence of chlorophylls from colorless organisms, it has been made. It is possible, of course, that some assumptions may not have been legitimate. For this reason the differentiating characters have in most instances been given in two parts. The first part includes those known characteristics, both positive and negative, which, in the author's opinion, most clearly define the genus. The second part includes those characteristics which have been assumed to be negative or are known to be negative and is introduced by the statement "do not or are not known to." The second part should not be regarded too lightly. The absence of any reference to the liquefaction of inspissated serum by numerous organisms led the author to the wrong assumption that they were possibly negative. The application of this general comparative approach to identification has revealed numerous organisms to be positive in this matter. This information has never been recorded, hence the alteration made at H, **93**. It is not impossible that other corrections may need to be made.

The material included in the Notes should not be regarded as unimportant to the generic description. In some cases characters relegated to this section are uniform throughout the genus but have not appeared in the manipulation of the Key. In the compilation of the data in the Notes the number of species described has been listed, and for individual tests the number positive over the number tested is also given. For example, there may be 82 species in the genus. The indole test may have been cited for 47 and may have been positive for 28. In the text these facts would be summarized as "indole produced 28/47." A perusal of these data will give an idea of the extent to which species in each genus have been uniformly examined.

A Guide to Study has been included to aid students in taxonomic studies.

References to the higher taxa has been generally avoided. This has been done purposely to encourage a reconsideration of the evidence at the generic level.

A COMPREHENSIVE KEY TO THE GENERA OF BACTERIA

USE OF THE KEY

First, determine the characters of the organism and then consult the Key, *always* commencing from the beginning. The Key poses a series of questions which can be answered in the affirmative or negative. Bold face numbers on the right hand side of the Key indicate the next number on the left to be consulted. The sequence should be followed until the right hand number is replaced by a generic name. Keys to the particular genus in the *Manual* should then be consulted for species identification.

1. Organisms green, blue-green or yellowish green, brown or red, containing chlorophyll "a" either in well defined chloroplasts or in the cytoplasm.............**Algae**

Organisms colorless or pigmented; if the latter, green pigments do not have the characteristics of chlorophyll "a".......... **2**

2. Diameter or width of cells exceeds 2.0 μ; proceed to............Section A p. 2

Diameter or width does not exceed 2.0 μ; proceed to.............Section B p. 7

Note: a. In the assessment of diameter or width, measurement must be made of the cells themselves and not of any capsular structures or sheaths which may surround them. In Section A some cells have widths up to 100 μ and are clearly visible to the naked eye.

2. b. To avoid confusion the following terms have been used in the sense indicated below (for comment on the use of them see the Introduction):

Multicellular Organism: a group of cells, arranged uniseriately, which are joined for the whole or major part of their width and result in an organism having a lateral wall with little or no indentation and lack of articulation at the septa. The septa are clearly visible in unstained cells after the removal of inclusion substances such as sulfur or fat.

In the Key, small organisms which appear to be unicellular in unstained preparations and in preparations stained by Gram but which are distinctly multicellular after being stained to show cell walls are treated with the unicellular organisms. A note to this effect appears at the appropriate place.

Unicellular Organism: an organism which shows no evidence of dividing septa, other than those involved in a normal division, in unstained preparations or in preparations stained to reveal cell walls.

Cell: a unicellular organism.

Filament: an elongated rod which shows no evidence of multicellularity without the application of special techniques designed to demonstrate cell walls.

Chain of Organisms: a group of unicellular or multicellular organisms, arranged uniseriately, which are completely separated or are attached for only a minor part of their width and are freely articulate at the points of attachment.

The presence or absence of a sheath should not be taken into consideration.

Trichome: this term as used in the Key may refer to (*a*) multicellular, nonflagellated, gliding organisms; (*b*) chains of unicellular, nonflagellated, gliding organisms; (*c*) nonmotile, multicellular organisms; and (*d*) multicellular flagellated organisms. These cover the different kinds of bacteria for which the term has been employed in descriptions in the *Manual*.

The presence of a sheath should not be taken into consideration.

Sheath: a hollow structure surrounding a chain of cells or a trichome. It may be close fitting but is not in intimate contact with the cells. Sheath-forming organisms usually produce a gum-like holdfast and a gum-like secretion resembling a capsule, but as the length of the chain or trichome increases, the gum-like secretion resolves into a hollow structure, the sheath, which lacks intimate contact with the cells.

Capsule: a substance secreted by microorganisms which forms an envelope around the cell and remains in intimate contact with it. Its margin may be sharply defined or, owing to its relative solubility in water, merge imperceptibly into the surrounding fluids.

SECTION A

1. Multicellular organisms with or without a sheath *or* unicellular organisms arranged in chains and surrounded by a sheath... **2**

Unicellular organisms................ **18**

2. Multicellular organisms not in a sheath **3**

Organisms in a sheath............... **11**

3. Both trichomes and abstricted cells are nonmotile and are not flagellated...... **4**

Either trichomes or abstricted cells or both are motile. Motility may be either by gliding on solid surfaces (nonflagellated) or by means of flagella. (Apparently nonmotile but flagellated cells are included in this section)........................... **5**

4. Trichomes up to 5000 μ in length attached basally by means of a globular holdfast; endospores produced in any or all cells within the trichome and obliquely situated; recorded from the alimentary canal of millipedes, cockroaches, and toads

Arthromitus p. 128
M p. 835

Long trichomes arranged in bundles; each cell contains one or more gas vacuoles which gleam reddish in transmitted light; do not deposit sulfur internally

Peloploca p. 81
M p. 270

Trichomes limited to four cells, the end
ones being rounded; may be arranged
chains; recorded from the buccal cavi
Simonsiella p. 1
M p. 8

5. Trichomes flagellated, peritrichous..
Trichomes or abstricted cells not flage
lated; motility of a gliding type on sol
surfaces or along adjacent cells........
6. Organisms approximately 5 μ in wid
and straight to curved; develop a large e
dospore apparently by fusion of several ce
within the trichome. The spore is normal
centrally located. Division of the organis
is preceded by formation of biconcave dis
within the trichome somewhat similar
those produced by the blue-green alga *O*
cillatoria; found in large numbers in th
cecum of the guinea pig
Oscillospira p. 12
M p. 83

Organisms 3 μ or more wide; actively m
tile; do not produce endospores; common
fresh dung....... **Caryophanon** p. 12
M p. 83

7. Spiral cells.. **Thiospirillopsis** p. 12
M p. 84

No spirals.........................
8. Entire trichomes are motile........
Entire trichomes are not motile; attache
by means of a holdfast; taper from th
base to the tip; single cells formed by ab
striction from the tip are motile by a glid
ing motion on a solid surface; trichome
characteristically arranged in rosettes bu
may occur singly; do not deposit sulfur internally............ **Leucothrix** p. 131
M p. 85

Entire trichomes are not motile; attached
by means of a holdfast; taper from the base
to the tip; single cells formed by abstric
tion from the tip are motile by a gliding
motion on a solid surface; trichomes characteristically arranged in rosettes but may
occur singly; deposit sulfur internally when
growing in sulfide-containing waters
Thiothrix p. 129
M p. 842

Note: Harold and Stanier (Bacteriol.
Revs., *19*, 1955, 49) were unable to find a

sheath on *Leucothrix* although the original description cited a prominent sheath. Pringsheim (Bacteriol. Revs., *21*, 1957, 69) reports that a prominent sheath does exist but cannot be demonstrated by means of nigrosin. It stains pink with methylene blue whereas the cells stain a dark indigo.

Winogradsky (Schwefelbacterien, Leipzig, 1888) also notes that the sheath of *Thiothrix* is almost indistinguishable in living specimens but becomes visible at the tip where hormogonia are separating or may be observed when degeneration occurs after removal of sulfide.

The insertion of *Leucothrix* and *Thiothrix* at this point in the Key is made to cover the possibility that sheaths may be overlooked.

9. Elemental sulfur is deposited in a globular form in the cells when growing in waters containing hydrogen sulfide
Beggiatoa p. 129
M p. 838

Elemental sulfur is not deposited internally **10**

10. Cells within the trichomes contain one or more gas vacuoles which gleam bluish or reddish in transmitted light; described from surface films in pond waters
Pelonema p. 81
M p. 271

Note: Pelonema is described as having a thin sheath and as possibly being motile. It seems unlikely that floating motile filaments would be ensheathed, and for this reason *Pelonema* is included here.

Not as above. . . . . . . **Vitreoscilla** p. 130
M p. 845

11. Width of sheath increasing from base to tip; cells within the sheath divide transversely and longitudinally towards the tip to produce large numbers of coccoid elements; attached by means of a holdfast. **12**

Width of the sheath uniform or variable; division of cells in transverse direction only **13**

12. Cells within the basal portion of the sheath longer than wide; when growing in iron-bearing waters, the sheath becomes heavily impregnated with iron
Crenothrix p. 82
M p. 272

Cell within the basal portion of the sheath much wider than long; sheaths remain colorless in iron-bearing waters
Phragmidiothrix p. 82
M p. 273

13. Cells within the base of the sheath 2 by 10 μ with rounded ends; divide transversely near the tip to produce spherical, nonmotile cells which are extruded either singly or in chains. The sheath becomes heavily impregnated with iron or manganese, becoming wide at the base and tapering towards the tip; attached by a holdfast; false branching is common
Clonothrix p. 82
M p. 274

Spirally wound to straight chains up to 250 μ long; sheaths heavily encrusted with iron
Leptothrix p. 80
(*L. pseudovacuolata*) *M p. 264*

Note: The single species may be a *Sphaerotilus*.

Not as above. **14**

14. Chains of cells enclosed in a sheath of uniform width; attached by means of a conspicuous holdfast; free cells motile by means of subpolar flagella
Sphaerotilus p. 79
M p. 263

Note: Species of *Sphaerotilus* have been shown to precipitate iron in the sheath, in which state they strongly resemble species of *Leptothrix*. Skerman, Dementjeva, and Carey (J. Bacteriol., *73*, 1957, 504) have shown that *S. natans* will also deposit sulfur internally. Although it has a superficial resemblance to *Thiothrix*, it differs in having flagella.

Not as above; if motile, not flagellated. **15**

15. Sulfur deposited internally when grown in water containing hydrogen sulfide. . . **16**

Sulfur not deposited internally. **17**

16. Several trichomes within a common sheath. . . . . **Thioploca** p. 129
M p. 841

A single trichome within each sheath; usually attached by a holdfast

Thiothrix p. 129
M p. 842

17. Colorless trichomes, attached at the base, tapering from the base to the tip; most characteristically arranged in rosettes but may occur singly. Constriction of the outer wall near the tips produces a beaded appearance. Single cells are abstricted and may exhibit a gliding motility on a solid surface. The trichomes themselves are immobile............. **Leucothrix** p. 131
M p. 850

Note: Harold and Stanier (Bacteriol. Revs., *19*, 1955, 49) were unable to find a sheath on *Leucothrix*, although the original description cited a prominent sheath. Pringsheim (Bacteriol. Revs., *21*, 1957, 69) reports that a prominent sheath does exist but cannot be demonstrated by means of nigrosin. It stains pink with methylene blue whereas the cells stain a dark indigo. Colorless trichomes up to 500 μ in length; each cell contains one or more gas vacuoles which gleam reddish or bluish in transmitted light; enclosed in a thin transparent sheath; occur singly... **Pelonema** p. 81
M p. 271

Note: Peloploca, which has a similar cellular morphology although described as "no sheath evident," and which occurs in bundles, should be compared carefully with *Pelonema.*

18. Spiral cells..................... **19**
Not as above........................ **23**

19. Cells contain bacteriochlorophyll and carotenoid pigments; cell masses various shades of red or purple.............. **20**
Not as above........................ **21**

20. Oxidize hydrogen sulfide, depositing sulfur internally... **Thiospirillum** p. 51
M p. 46

Do not oxidize hydrogen sulfide

Rhodospirillum p. 54
M p. 58

21. Rigid cells 6 to 50 μ long; actively motile by means of polar flagella; deposit sulfur internally when growing in waters containing hydrogen sulfide

Thiospira p. 5
M p. 8

Flexible cells; not flagellated; do not d
posit sulfur internally.............. 2

22. Large, spiral cells with tapered end
up to 100 μ long; protoplast wound spirall
around a well defined axial filament; n
cross striations; motile by means of a fle
uous movement.... **Spirochaeta** p. 13
M p. 89

Spiral cells with a round cross section an
blunt ends; up to 60 μ long; cells have
ridge or crista composed of numerous f
brils running along one side of the spira
cross striations distinct; found in the in
testinal tract of molluscs

Cristispira p. 13
M p. 89

23. Stalked cells; aquatic in habit..... 2
Cells not borne on stalks............ 2

24. Cells rod-shaped; 2 by 6 to 12 μ; singl
cells attached terminally and at right angle
to branches of a lobose, dichotomousl
branched stalk; form globular bush-like o
plate-like growths on the surface of wa
ters.................. **Nevskia** p. 8
M p. 21

Cells pear-shaped to spherical; multiply b
budding; cells attached by a long slende
stalk to a holdfast with several stalks fre
quently arising from one holdfast. (Thi
organism has so far been found only in lak
waters where the temperature does not ex
ceed 23° C.)........ **Blastocaulis** p. 8
M p. 27

Cells pear-shaped; borne on a very shor
stalk; cells grow attached to each other in
cauliflower-like mass and reproduce b
longitudinal division and budding. Colo
nies break up at intervals, and liberate
cells start new colonies. Cells and method
of reproduction resemble those found i
Chaemosiphon, a blue-green alga; discov
ered in the body cavity of fresh water crus
taceans............. **Pasteuria** p. 8
M p. 27

25. Endospores produced........... 2
No endospores produced............ 2

26. Spherical cells in cubical packets
Sarcina p. 101
M p. 467

Rod-shaped cells... **Clostridium** p. 116
M p. 634

27. Cells contain bacteriochlorophyll and carotenoid pigments; cell masses are various shades of red, brown, and purple; proceed to............... Section J p. 43

Not as above...................... **28**

28. Iron deposited on the cells or in capsules............................ **29**

Note: In the absence of further information, these organisms are identified on their iron-depositing characteristics. Most iron organisms studied in pure culture metabolize the organic compound which forms the iron chelate, and the liberated iron chelates with some cell component. Citrate-utilizing organisms will, for example, release iron from ferric ammonium citrate. Accumulation of the iron in or on the cell may depend only upon the nature of the cell substance. Pure culture studies may place these organisms in more commonly recognized genera. Many more organisms, if tested, may fall into the following genera. They should also be treated as non-iron-depositing cells and should be followed through the Key.

Not as above...................... **32**

29. Iron deposited as a torus, a solid ring partially or completely surrounding the cell in one area only, giving the cells the appearance of open or closed links of a chain **30**

Iron deposited uniformly over the cells or capsules......................... **31**

30. Cells completely surrounded by a torus
Naumanniella p. 69
M p. 223

Cells only partially enclosed, appearing like a horseshoe. Flagella of unequal length borne at the open end
Ochrobium p. 69
M p. 225

Note: The type of flagellation suggests that this may be an alga.

31. Spherical cells 1 to 2 μ in diameter, 2 to 60 or more cells occurring in a primary capsule 10 to 20 μ wide; secondary capsules unite to form a mucilaginous colony; iron or manganese compounds are stored in the secondary capsules.. **Siderocapsa** p. 67
M p. 218

Cells coccoid to ovoid, 4.8 to 5.0 by 6.5 μ, forming short chains embedded in a thin mucilaginous layer; iron compounds stored in the surface membrane of the cells
Sideronema p. 68
M p. 220

Cells rod-shaped, 2.5 by 6 to 15 μ, straight or slightly bent; not encapsulated; iron or manganese stored on the surface or in the membrane of the cell
Siderobacter p. 69
M p. 226

32. Strict intracellular parasites occurring in the cytoplasm of conjunctival cells in cattle, goats, and sheep. Elliptical, coccoid, rod-shaped, and comma-shaped cells occur
Colettsia p. 40
M p. 961
See also Section K p. 45

Spherical cells produced in macroscopic fruiting bodies on decaying vegetable material or in culture; fruiting bodies sessile or nearly so. The cocci germinate to produce rod-shaped cells which glide on a solid surface; not flagellated
Myxococcus p. 135
M p. 883

Or........... **Chondrococcus** p. 135
M p. 886

See Section L for criteria for separation. p. 135

Pleomorphic cultures consisting of large and small cocci and small rod-shaped cells which are motile by means of a single polar flagellum; strongly halophilic, requiring 20 to 30 per cent salt for optimal growth; Gram-negative... **Halobacterium** p. 66
(*H. cutirubrum*) *M p. 207*

Not as above...................... **33**

33. Cells spherical to ovoid, varying from spheres 5 μ in diameter to large cylindrical organisms 35 to 100 μ long; sulfur deposited internally when growing in the presence of hydrogen sulfide. In one of the two recorded

species, large crystals of calcium carbonate fill the cells; motile with a slow jerky rotating action when in contact with solid surfaces......... **Achromatium** p. 131
M p. 852

Cells spherical to ovoid, 5 to 20 μ in diameter, with the cytoplasm compressed in one end of the cell; sulfur deposited in the cytoplasmic layer; exhibits an extremely rapid darting motion in free solution suggestive of flagella, which have never been demonstrated; found in waters containing hydrogen sulfide, forming a tenacious web-like growth in a zone of critical hydrogen sulfide-oxygen concentration
Thiovulum* p. 59
M p. 81

Not as above...................... **34**

34. Cocci varying in diameter from 0.5 to 4.0 μ; grow in a mineral salts-bicarbonate medium with formate as the only known source of available carbon, fermenting it to methane, CO_2, and possibly hydrogen; pH range, 7.4 to 9.2. **Methanococcus** p. 102
(*M. vannielii*) *M p. 473*

Not as above...................... **35**

35. Spherical cells.................. **36**

Rods, curved or straight........... **43**

36. Arranged in cubical packets
Sarcina p. 101
M p. 467

Not as above...................... **37**

37. Motile by means of peritrichous flagella............................ **38**

Nonmotile........................ **40**

38. Gram-positive; cells occur in irregular clusters........... **Micrococcus** p. 99
(*M. cryophilus*) *M p. 455*

Gram-negative.................... **39**

39. Cells coccoid only at pH 7.0 on peptone yeast extract acetate agar; develop into multicellular rods with peritrichous flagella under other conditions; do not fix atmospheric nitrogen... **Caryophanon** p. 127
M p. 831

Cells grow in nitrogen-free mineral salts media containing a suitable source of carbon, fixing atmospheric nitrogen
Azotobacter p. 8
M p. 2

40. Aerobic........................ 4
Anaerobic......................... 4

41. Gram-positive; cells occur in irregul
clusters........... **Micrococcus** p. 9
M p. 4

Gram-negative; fix atmospheric nitroge
The coccoid form is only part of a cycle
morphological forms, the initial stage
which is a large rod.. **Azotobacter** p. 8
M p. 28

42. Large cocci, 3 to 4 μ wide, sometim
bearing rod-shaped protuberances on o
posite sides and at an obtuse angle to on
another—a pleomorphic stage of a ro
shaped cell 0.8 by 2.4 to 10 μ; produ
copious gas from peptone
Sphaerophorus p. 9
(*S. ridiculosis*) *M p. 44*

Spherical cells; pleomorphic, ranging in d
ameter from 0.7 to 2.5 μ; occurring in pair
short chains, and in irregular groups; de
pendent upon glycine for growth in organi
media. Glycine is decomposed to CO_2
NH_3, and acetic.. **Peptococcus** p. 10
(*P. glycinophilus*) *M p. 47*

43. Large, cylindrical, pear-shaped o
slightly curved rods 3 to 14 μ wide; ac
tively motile by means of a single pola
flagellum; contain large spherules of cal
cium carbonate and may also contain sul
fur.............. **Macromonas** p. 5
M p. 8

Not as above...................... 4

44. Curved rods................... 4

Straight rods...................... 4

45. Curved rods with a bunch of flagell
inserted laterally in the concave part of th
cell; anaerobic; recorded from the cecum
of the guinea pig, the buccal cavity of man
and the rumen of the herbivore
Selenomonas p. 7
M p. 25

Curved rods with polar flagella; 1.7 to 2.
by 6.6 to 14.0 μ; contain small globules o
sulfur in the center of the cell and a singl

---

* See notes on *Thiovulum*, p. 59

large volutin granule at each end
Thiospira p. 59
M p. 82

46. Cells 1.4 to 2.0 by 4.0 to 5.0 μ; motile by means of polar flagella; anaerobic to microaerophilic; ferments glucose, producing ethyl alcohol, carbon dioxide, and lactic acid.............. **Zymomonas** p. 65
M p. 199

Motile by means of peritrichous flagella; grow in a nitrogen-free mineral salts medium, fixing atmospheric nitrogen
Azotobacter p. 84
M p. 283

SECTION B

Note: Criteria for the separation of the small, colorless flagellated protozoan forms from bacteria are very limited. Organisms which (*a*) when stained with Giemsa stain show a clearly differentiated nucleus and cytoplasm without preliminary acid hydrolysis, (*b*) divide along the longitudinal axis, and (*c*) possess flagella or cilia which are clearly discernible without staining are possibly protozoa. A cross section of the flagella of protozoa examined with the electron microscope shows a structure quite unlike that of bacterial flagella. The flagella of protozoa consist of a pair of central fibrils surrounded by nine pairs of peripheral fibrils all enclosed in a sheath (C. K. Pine, Exptl. Cell Research, *14*, 1958, 388).

1. Ultramicroscopic and filterable forms; strict intracellular parasites of animals and plants not cultivable on artificial media but transferable by contact or by arthropod vectors........................ **Viruses**

2. Strict parasites occurring within tissue cells of animal hosts or on or in erythrocytes. With few exceptions, which have been treated under Section H, they cannot be or have not been cultivated in artificial media. Some can be cultivated in chick embryos or in tissue cultures. In the tissues or blood stream they occur *either* as spherical elementary bodies and initial bodies from 0.2 to 2.0 μ in diameter or slightly larger (usually 0.20 to 0.35 μ), singly or in aggregations in plaques several microns in diameter *or* as bacillary, triangular, ring-shaped, horseshoe-shaped, and other pleomorphic forms. Bacillary forms may be as long as 3 μ. Stain with Giemsa's or Macchiavello's stain without differentiation into cytoplasmic and nuclear structures, a condition which would be suggestive of protozoa.................. Section K p. 45

3. Small, spherical bodies, 150 to 300 mμ in diameter, which germinate to produce filaments approximately 0.2 μ wide and from 2 to 50 μ long, sparsely or richly branching. At a later stage of growth small endomycelial corpuscles develop in the filaments by a process of successive condensation and constriction. As a result the homogeneous filaments are retransformed into chains of close-set spherical bodies which are released by fragmentation; highly resistant to penicillin and sulfathiazole; colonies on agar have a dense granulated central area which penetrates into the agar and which is surrounded by a translucent, flat, peripheral zone or consist of a pearly film containing numerous spots due to calcium or magnesium soaps; do not ferment lactose, sucrose, mannitol, or dulcitol
Mycoplasma p. 138
M p. 914

Note: L-phase colonies of some bacteria bear a strong resemblance to the colonies of *Mycoplasma*. They are generally more opaque, more heavily marked on the surface, tend to revert to the normal bacillary form in penicillin-free, semisolid media, are more difficult to subculture, do not require cholesterol for growth, and ferment the same carbohydrates as the parent organism.

4. Spiral cells; proceed to
Section C p. 9

This section does not include (*a*) all forms like *Vitreoscilla*, which, through their great length and extreme flexibility, are apt to coil in one plane in watch spring fashion; (*b*) spiral cells of the streptomyces type which arise from branching

Gram-positive filaments; or (*c*) chains of vibrios. The latter do not possess the true helical twist of the spiral organisms.

5. Spherical to ovoid cells which reproduce by production of a tubular outgrowth, 0.2 to 0.3 μ wide, from the cell on the end of which a daughter cell is formed. The tubular outgrowths may be simple or branched. Daughter cells are initially spherical but are later ovoid to rod-shaped; colorless or contain photosynthetic pigments.

Colorless cells, ovoid, 0.5 by 1.0 μ when mature; motile by means of a single polar flagellum; daughter cells may break loose from the tubular outgrowth and form tubes of their own while still actively motile

Hyphomicrobium p. 55
M p. 277

Cell masses salmon pink to a deep orange-red; cells ovoid, 1.2 by 2.8 μ; nonmotile; contain photosynthetic pigments; grow only under anaerobic conditions when exposed to light.. **Rhodomicrobium** p. 55
M p. 277

6. Spherical cells which reproduce by binary fission or by budding. Well defined stalks are secreted by some species, the budding form of reproduction being confined to the stalked types; proceed to

Section D p. 11

See also Section B, **10**.............. p. 8

7. Vegetative cells, rod-shaped, not spirally twisted; Gram-negative. Microcysts produced in macroscopically visible fruiting bodies or occur loosely among elongated S-shaped, twisted or straight, flexible Gram-negative rods; germinate to produce rod-shaped cells which are motile only by a creeping action on solid surfaces. These rods may contract to form spherical microcysts or may combine in groups to form fruiting bodies in which the spherical or rod-shaped microcysts are formed

Section L p. 47

8. Rod-shaped cells, 0.5 to 1.5 by 2 to 5 μ, which grow in colonies on the surface of water containing sulfide and which deposit sulfur either inside or outside the cells. One species forms bladder-like gelatinous colonies with the bacteria embedded in t surface......... **Thiobacterium** p.
M p.

Note: This very poorly defined group separated here because of a complete la of information of other properties. It is su gested that any such forms, if found, shou be keyed out in the section on rods to d termine their possible taxonomic relatio ship. The presence of the sulfur around t cells in such a location may not be signi cant.

9. Rod-shaped and filamentous forms r producing by binary fission, by fragmenta tion of the mycelium, by the production endospores or conidia or by the productio of microcysts; proceed to

Section E p. 1

10. Colorless, spherical cells 0.8 to 1.0 in diameter, arranged in parallel rows i flat sheets on the surface of liquid manur and culture media. The sheets break char acteristically into squares, each of 16 cells Grows well on acetate plus beef extract plu yeast extract agar. Single cells are rare

Lampropedi

Note: This genus does not appear in th seventh edition of the *Manual.* The de scription given by Pringsheim (J. Gen Microbiol., *13*, 1955, 285) does not give clear indication of the shape or size of th cells, emphasis being placed on the peculia colony form of which an excellent illustration has been given. The information o morphology given above was obtained b phase contrast examination of a cultur kindly supplied by Dr. Pringsheim. In preparations heat-fixed and stained by Gram the cells are Gram-negative and appear t be lenticular in shape.

11. *Note:* Attention is drawn to a new group of microorganisms described by A. E. Kriss and I. N. Mitzkevich (J. Gen. Microbiol., *20*, 1959, 1) in a paper entitled "Krassilnikoviae: A New Class of Microorganisms found in the Sea and Ocean Depths". The authors give the following diagnosis: "filaments, non-septate, nonramified, diameter 0.4–0.5 μ may be enclosed in a sheath. At one end form a cluster-shaped head, consisting of rounded bodies of diameter 0.5–2.0 μ. The number of round bodies in a cluster on one filament may amount to several scores. The organisms are widely spread in seas and oceans. May be found in considerable number at deep horizons. Have not been obtained in laboratory culture. Do not grow in conventional media or in isolated sea water samples. Rapidly develop on submerged fouling slides."

SECTION C

1. Organisms contain chlorobium chlorophyll *or* bacteriochlorophyll with carotenoid pigments 2
Organisms do not contain photosynthetic pigments 4
2. Nonmotile cells containing only chlorobium chlorophyll; appear distinctly green even under a microscope; may be found in *pure cultures* associated with other morphological forms such as rods and streptococcal forms with the latter often predominating; strictly anaerobic cells which oxidize sulfide, depositing sulfur outside the cells
Chlorobium p. 52
M p. 62

Note: This pleomorphism, recorded by van Niel, has been disputed by later investigators.

Cells contain bacteriochlorophyll and carotenoid pigments; red or purple in masses of cells; actively motile by means of polar flagella 3
3. Organisms grow autotrophically under anaerobic conditions exposed to light; oxidize sulfide and thiosulfate to sulfur, which is deposited inside the cells
Thiospirillum p. 51
M p. 46

Organisms will grow anaerobically when exposed to light but will not grow under strictly autotrophic conditions; require growth factors available in yeast extract; may oxidize sulfide but do not oxidize thiosulfate; sulfur is not deposited in the cells
Rhodospirillum p. 54
M p. 58

4. Uniseriate chains of cells enclosed in a sheath; impregnated with iron when in iron-bearing waters; spirally wound around themselves or algal filaments
Leptothrix p. 80
M p. 264

Note: Species of *Sphaerotilus*, considered by Pringsheim (Phil. Trans. Roy. Soc. London, Ser. B, *233*, 1949, 453) and others as identical with *Leptothrix*, frequently show spirally twisted, sheathed forms among normally straight ones.

Chains of curved rods wound into a ball within a nearly spherical capsule; do not store iron or manganese
Myconostoc p. 73
M p. 260

Spiral cells bearing a torus of iron hydroxide
Naumanniella p. 69
M p. 223

Very thin cells wound into tight cylindrical coils, 15 to 20 μ long; may be embedded in a capsular material when grown on silica gel. Slowly oxidize ammonia to nitrite
Nitrosospira p. 56
M p. 70

Not as above 5
5. Nonmotile trichomes spirally wound around each other in bundles; not ensheathed; cells within the trichomes contain gas vacuoles which have a reddish gleam in transmitted light
Pelploca p. 81
M p. 270

Motile trichomes having a slow, creeping, rotating type of motility on solid surfaces with the tips of the filaments oscillating; no flagella; deposit sulfur internally from sulfide-containing waters
Thiospirillopsis p. 129
M p. 840

Not as above 6
6. Cells parasitic on the protozoan, *Paramecium* 7
Not as above 8
7. Cells contain 1.5 to 2.5 spiral turns;

tapered at the ends; parasitic within the micronucleus of *Paramecium aurelia*, causing marked enlargement of the micronucleus, which is filled with spirals

Holospora
(*H. undulata*) *M p. 929*

Cells twisted in two spiral turns that are not abrupt; one end pointed and the other rounded; no flagella; movement helicoid; endospores are formed; parasitic in the cytoplasm of *Paramecium caudatum*

Drepanospira
M p. 928

8. Cells, not more than 10 μ long, which may appear spiral in heat-fixed and stained preparations; flexible rods in the living state; motile by means of a creeping action on solid surfaces

possibly **Cytophaga** p. 132
M p. 858

See also Section L................ p. 47

Not as above; cells actively motile in free solution............................ **9**

9. Cells relatively rigid; motile by means of polar flagella..................... **10**

Cells flexible; motile by means of a helicoid flexing action: no flagella............. **12**

10. Cells oxidize hydrogen sulfide, depositing sulfur as small globules in the center of the cell with volutin granules towards the ends............. **Thiospira** p. 59
M p. 82

Not as above...................... **11**

11. Cells consisting of a single complete spiral twist; rather sharply angulated; cells 1.5 to 2.0 μ wide at the center and tapering towards both ends. In the center of the cell is an ovoid to rounded body almost as wide as the cell and clearly visible without staining. It stains deeply with neutral red in killed cells and with Heidenhain's iron-hematoxylin or Giemsa's stain in fixed cells and is considered to be a nucleus; motile by means of polar flagella; when attached to an object at one end, cells are capable of contraction to a more angulated spiral

Paraspirillum p. 73
M p. 257

Note: The author has seen in pond waters several cells very similar morphological to these cells except that the central bod appeared pale green and may be a chlor plast.

Not as above; spirals quite rigid; mov ment of a definite helical type in liquids

Spirillum p. 7
M p. 25

Note: Several species of vibrios (*V. sput genus*, *V. jejuni*, *V. coli*, *V. indicus*, *V luminosus*, and *V. marinopraesens*) are de scribed as forming spiral chains. If a vibri is curved along only one axis, formation c a true spiral is not possible. Cells forming true spiral when in chains must have th basic helical twist in the axis of the indi vidual cells and should be classified a *Spirillum.*

Reference should be made to the monc graph by Williams and Rittenberg (Intern Bull. Bacteriol. Nomen. and Taxon., 7 1957, 49) for a revised classification of th spirillae.

12. Cells 20 to 300 μ long and 0.25 to 2.0 wide (generally 0.25 to 0.5 μ) with the pro toplast wound around a well defined axia filament; cells very flexible and activel motile; flagella absent; fresh water and sal water forms....... **Spirochaeta** p. 13
M p. 89

Spiral cells, 0.5 to 3.0 μ wide and 10 to 10 μ long, with a spiral amplitude of 6 to 8 μ flexible cells characterized by a thin membrane or crista on one side of the body which extends the entire length of the cell cross striations in stained cells are distinct actively motile without flagella; recorded from the crystalline style sac in the alimentary canal of molluscs

Cristispira p. 136
M p. 895

Spiral cells, 0.5 to 1.2 μ wide and 60 to 80 μ long, with a spiral amplitude of 4 to 25 μ; cross striations in stained cells distinct; no axial filament or crista; actively motile but not flagellated; found in oysters and also free living......... **Saprospira** p. 136
M p. 894

Not as above...................... **13**

13. Not readily stained; stain with Giemsa's stain or by silver impregnation methods; visible unstained under dark ground but rarely by ordinary light microscopy **14**

Cells stain readily; Gram-negative; cells rarely more than 1 μ wide; spirals frequently irregular and of variable amplitude
Borrelia p. 137
M p. 897
Treponema (T and W, 4th ed.)

14. Aerobic; cells 0.1 to 0.2 μ thick; wound in a very fine coil and hooked at one or both ends; can be cultivated *in vitro* in semisolid rabbit plasma media
Leptospira p. 137
M p. 907

Anaerobic; very fine coiled cells of uniform amplitude; may be pointed at both ends but not hooked; not yet cultivated *in vitro*
Treponema p. 137
M p. 904

SECTION D

1. Organisms contain photosynthetic pigments alone or with carotenoid pigments. Cells in masses appear green to greenish yellow or red to purple. These organisms will grow in certain media under anaerobic conditions only when exposed to light. Certain species are also capable of aerobic growth in the dark **29**

Not as above **2**

2. Single cells borne on the end of elongated stalks; aquatic forms **3**

Cells not borne on stalks **4**

3. Stalks band-shaped and twisted into a flat spiral; dumbbell-shaped in cross section; composed entirely of or impregnated with ferric hydroxide; dissolves completely in dilute mineral acids; a single cell is borne at the end of each stalk
Gallionella p. 83
p. 214

(See Note on *Gallionella*, p. 83.)

Stalks long and slender; attached to some solids object by means of a holdfast; cells spherical to pear-shaped; reproduce by budding, with the daughter cells subsequently secreting individual stalks
Blastocaulis p. 84
M p. 279

4. Cells or their capsules impregnated with iron or manganese **5**

Note; In the absence of further information, these organisms are identified on their iron-deposting characteristics. Most iron organisms studied in pure culture metabolize the organic compound which forms the iron chelate, and the liberated iron chelates with some cell component. Citrate-utilizing organisms, for example, will release iron from ferric ammonium citrate. Accumulation of the iron in or on the cell may depend only on the nature of the cell substance. Pure culture studies may place these organisms in more commonly recognized genera. Many more organisms, if tested, may fall into the following genera. They should also be treated as non-iron-depositing cells and should be followed through the Key.

Not as above **7**

5. Not encapsulated
Siderococcus p. 69
M p. 225

Cells encapsulated **6**

6. Groups of cocci arranged in pairs in a common capsule
Siderosphaera p. 68
M p. 220

Cocci occur singly or in unordered groups in a common capsule . . **Siderocapsa** p. 67
M p. 218

7. Cells coccal only as a stage in a definite life cycle *or* as a pleomorphic phase of rod-shaped bacteria **8**

Cells coccal at all stages of growth **15**

8. Cells have a definite cyclic form of development; spherical cells germinate at one or more points to produce rod-shaped cells which elongate and divide. At the point of division, growth of the cells continues at an angle to the original axis. When the side branch is equal in size to the parent cell, division occurs at the angle. This process is repeated during the growth of the colony. *In older colonies, the rods transform entirely*

into a mass of cocci. Rods are most frequently Gram-negative with Gram-positive granules; cocci are frequently Gram-positive; soil inhabitants
Arthrobacter p. 114
M p. 605

Note: The emphasis lies on the final transformation into cocci. Some authorities may consider that limited true branching may occur. If this is admitted, the dividing line between *Arthrobacter* and *Nocardia* becomes very slim. The author's observations of *Arthrobacter globiformis* fit the above statement, and it is suggested that these criteria be adopted, with true branching forms which later disintegrate being assigned to *Nocardia.*

Not as above **9**

9. Gram-positive **10**

Gram-negative **11**

10. Organisms occur as cocci under anaerobic conditions in neutral media; in media becoming acid, they assume a diphtheroid form; extremely pleomorphic under aerobic conditions; *produce propionic acid from lactic acid* . . **Propionibacterium** p. 110
M p. 569

Not as above
possibly **Mycococcus** p. 119

Note: The description of *Mycococcus* has a strong resemblance to that of *Arthrobacter* in the early literature, and the two generic names may be synonyms. *Micrococcus cinnabareus* and *M. rhodochrous* are included here.

11. Anaerobic; nonmotile; Gram-negative; principally rod-shaped cells exhibiting a coccoid phase; recorded from genital and alimentary tracts of man and other animals . . . . **Sphaerophorus** p. 97
M p. 441

Note: The type species of this genus is listed as *Fusiformis necrophorus* in Topley and Wilson.

Aerobic **12**

12. Obligate halophiles requiring 16 to 30 per cent salt for growth; not luminescent
Halobacterium p. 66
M p. 207

Not obligate halophiles . . . .

13. Parasites attacking erythrocytes a
endothelial cells of man; extremely ple
morphic within the host; straight a
curved rods, ring forms, and cocci occu
grow in semisolid rabbit serum agar main
as rods and cocci; polar flagella
Bartonella p. 1
M p. 9

Note: This genus is selected as an examp
only of a large group of intracellular par
sites which are pleomorphic and have cocc
stages. Only an odd species has been cult
vated. For other genera see Section K
p.

Animal parasites; nonmotile; produce tu
laremia or tularemia-like infections in r
dents . . . . **Pasteurella** p. 7
M p. 3

Note: Pasteurella tularensis is listed a
Brucella tularensis in Topley and Wilson.

Organisms occurring as diplococci or pr
mary isolation on solid media; give rise to
proportion of rod-shaped oranisms in liq
uid which increases as the culture ages. Ro
forms may show bipolar staining and ar
capsulated:

(i) no acid from carbohydrates
Mima p. 7

(ii) acid or acid and gas from carbo
hydrates

(a) acid from carbohydrates
Herellea p. 7

(b) acid and gas from carbohydrate
Colloides p. 7

Note: The genera *Mima*, *Herellea*, and
Colloides do not appear in the *Manual*
They are described by de Bord (Iowa Stat
Coll. J. Sci., *16*, 1942, 471) and have been mentioned by a number of authors in connection with a species described as *Bacterium anitratum.* They appear to merit consideration. Isolates were obtained from conjunctival and vaginal specimens.

Not as above **14**

14. Bioluminescent when grown on fish agar or meat infusion agar containing 3 per cent salt . . . . **Photobacterium** p. 64
M p. 19

Organisms not fitting into any of the above groups are probably pleomorphic forms of od-shaped cells; proceed to
Section E p. 14

15. Organisms parasitic within the cytoplasm or nucleus of flagellated protozoa. **16**

Not as above.......................... **17**

16. Parasitic on the nucleus or nucleolus
Caryococcus *M p. 927*

Parasitic in the cytoplasm of *Trichomonas batrachorum*
Micrococcus (*M. batrachorum*) *M p. 929*

17. Strict autotrophs; will not grow on organic media; oxidize ammonia to nitrite, or nitrite to nitrate.................... **18**

Heterotrophic...................... **19**

18. Oxidize ammonia to nitrite; nonmotile; not encapsulated...**Nitrosococcus** p. 56
M p. 69

Other than above; proceed to
Section G p. 102

19. Obligate anaerobes.............. **20**

Aerobic or microaerophilic........... **23**

20. Gram-variable; cells occur singly or in masses; ferment acetate vigorously with the production of methane; discovered in mud
Methanococcus p. 103
M p. 473

Not as above...................... **21**

21. Gram-positive.................. **22**

Gram-negative; arranged in pairs and clusters; diameter of cells varies among species from 0.3 to 2.0 μ....**Veillonella** p. 103
M p. 485

22. Organisms arranged in pairs or chains only.......**Peptostreptococcus** p. 106
M p. 533

Note: This genus includes organisms treated under the general heading of 'anaerobic streptococci' by Topley and Wilson.

Organisms arranged in cubical packets
Sarcina p. 101
M p. 467

Organisms arranged singly, in pairs, and in irregular clusters; rarely in chains
Peptococcus p. 102
M p. 474

Note: Peptococcus constellatus may belong to *Peptostreptococcus.* The species of this genus are referred to under the general heading of 'anaerobic micrococci' by Topley and Wilson.

23. Gram-negative, kidney-shaped to hemispherical cells occurring basically in pairs with the flat sides adjacent; animal parasites.................**Neisseria** p. 102
M p. 480

Note: a. *Micrococcus morrhuae* and *M. roseus* are described as Gram-negative and as occurring singly and in pairs.

b. Compare with the genera *Mima*, *Herellea*, and *Colloides* at D, **13**.

Not as above...................... **24**

24. Organisms arranged in cubical packets
Sarcina p. 101
M p. 467

Organisms from liquids are arranged singly, in pairs or as tetrads, and occasionally in short chains; tetrads common in acid media; produce at least 0.5 to 0.7 per cent acid in yeast extract-glucose-tryptone-phosphate broth, lowering the pH below 4.0; *lactic acid produced is optically inactive*; found in fermented liquids and foods
Pediococcus p. 105
M p. 529

Not as above...................... **25**

25. Organisms arranged in pairs only or in chains of varying lengths when growing in liquid media...................... **26**

Organisms arranged singly, in pairs and in clusters in liquid media; occasional species are motile........................ **28**

26. Organisms produce gas in Eldredge tubes when growing in yeast extract-glucose-tryptone-phosphate broth; *lactic acid produced is levorotatory*; frequently produce a copious gum (dextran) in sucrose broth
Leuconostoc p. 106
M p. 531

Not as above; lactic acid produced is *dextrorotatory*........................ **27**

27. Parasitic; cells usually in pairs, particularly in pathological material, in which they are encapsulated; chains are common in culture media; bile-soluble; found in the

respiratory tract of man
Diplococcus p. 104
M p. 507
Streptococcus (T and W, 4th ed.)
Other than above; parasitic or saprophytic
Streptococcus p. 105
M p. 508

28. Cells occur predominantly as tetrads and are encapsulated in body fluids; occur as tetrads and in irregular masses in cultures.................**Gaffkya** p. 101
M p. 466
Micrococcus (T and W, 4th ed.)
Not as above......**Micrococcus** p. 99
M p. 455
Staphylococcus p. 100
M p. 464

Note: a. Placed in the genus *Staphylococcus* are two species, *Staphylococcus aureus* and *Staphylococcus epidermidis.* Reference should be made to the species descriptions for separation from the other micrococci.

b. Williams, Hirch, and Cowan (J. Gen. Microbiol., *8*, 1953, 475) have suggested the separation of a new genus, **Aerococcus** from the other micrococci because of the ability of these organisms to grow in the presence of 40 per cent bile and their production of a pronounced greening around the colonies on blood agar. See p. 100.

See also Section B, **10.**

29. Cell masses are green or yellowish green; probably contain chlorobium chlorophyll and not bacteriochlorophyll..... **30**
Cell masses red, brown, or purple; proceed to....................Section J p. 43

30. Spherical to ovoid cells occurring in chains and forming flat sheets in which the chains are parallel. Oxidize hydrogen sulfide but do not store sulfur inside the cells
Pelodictyon p. 53
M p. 63
Spherical cells united in loose trellis-like aggregates. Sulfur is deposited internally
Clathrochloris p. 53
M p. 64

SECTION E

1. Multicellular organisms (sheathed or n
sheathed) *or* chains of unicellular organis
enclosed in a sheath.................

Note: This does not include chains
individually encapsulated cells or cells
zoogloeal masses.

Not as above; proceed to
Section F p.

2. Organisms enclosed in a common shea

Not as above.......................

3. Width of the sheath increasing from th
base to the tip; cells within the sheat
divide transversely and longitudinally t
wards the tip to produce large numbers
coccoid elements; attached by means of
holdfast.........................
Not as above........................

4. Cells within the basal portion of th
sheath longer than wide; when growing i
iron- or manganese-bearing waters, becom
heavily impregnated with iron or ma
ganese..............**Crenothrix** p. 8
M p. 27
Cells within the basal portion of the sheat
are wider than long; no iron deposited
Phragmidiothrix p. 8
M p. 27

5. Sheaths become impregnated with iro
or manganese when growing in iron- o
manganese-bearing waters or media....
Not as above.......................

6. Organisms in which the sheaths spli
longitudinally into fine, hair-like sections
The chain of cells remains attached to th
hairs at several points and, with continue
growth, causes arching of the hairs and o
the chain of cells within its new sheath, re-
sulting in the formation of a helm-like mas
Toxothrix p. 8
M p. 26
Not as above.......................

7. Cells within the base of the sheath 2 b
10 μ, with rounded ends; divide trans-
versely near the tip to produce spherical
nonmotile cells which are extruded eithe

gly or in chains. The sheath is heavily pregnated with iron or manganese, be- ming much wider at the base and taper- g towards the tip; attached by a hold- st; false branching is common

Clonothrix p. 82
M p. 274

the absence of iron, cells within the eath are of uniform size except during vision. During deposition of iron in the eath, cells in the more heavily impreg- ted areas become much narrower than rmal; extruded cells are of the same di- ensions as those within the sheath

Leptothrix p. 80
M p. 264

Note: Species of *Sphaerotilus* will also be und to terminate at this point if grown ferric ammonium citrate media. The enera are considered to be identical by ringsheim (Phil. Trans. Roy. Soc. Lon- on, Ser. B, *233*, 1949, 605) and others, but is relationship has been disputed by eger and Bringmann (Zentr. Bakteriol., arasitenk., Abt. II, *107*, 1953, 318).

. Endospores produced within the cells of he trichome and located in an oblique osition............**Coleomitus** p. 128
M p. 836

o endospores..................... **9**

. Organisms oxidize hydrogen sulfide, de- ositing sulfur inside the cells........ **10**

rganisms do not oxidize hydrogen sulfide nd do not deposit sulfur internally.. **11**

0. Several trichomes within a sheath

Thioploca p. 129
M p. 841

single trichome in each sheath

Thiothrix p. 129
M p. 842

Note: The author has demonstrated that *phaerotilus*, when exposed to hydrogen ulfide, deposits sulfur internally. Super- icially such cells show a very striking re- emblance to illustrations of *Thiothrix*. owever, the isolated cells of *Sphaerotilus* re motile with subpolar flagella whereas those of *Thiothrix* are nonflagellated and exhibit a gliding motility.

11. Single cells in chains within a common sheath; free cells motile by means of subpolar flagella or nonmotile

Sphaerotilus p. 79
M p. 263

(See note on *Leptothrix* and *Thiothrix* (vide supra **7** and **10**).)

Trichomes........................ **12**

12. Trichomes attached; free cells formed by abstriction from the terminal portion are motile by a gliding action on a solid surface.............**Leucothrix** p. 131
M p. 850

Note: Although Harold and Stanier claim that *Leucothrix* does not form a sheath, they consider it to be identical with *Pontothrix*, which was originally recorded as a sheathed organism.*

Not as above; if motile, whole trichomes move; cells within the trichomes each contain one or more pseudovacuoles which give a reddish gleam in transmitted light **13**

13. Trichomes occur singly

Pelonema p. 81
M p. 271

Trichomes occur in bundles

Peloploca p. 81
M p. 270

Note: Peloploca is described in the *Manual* as "sheath not demonstrated." It is included here on the possibility that it is sheathed and because of the morphological similarity to *Pelonema*.

14. Trichomes 6 to 20 μ long; motile by means of peritrichous flagella; rigid, multicellular, bacillary forms in which the stained cells are differentiated into a series of light and dark bands; end cells rounded; commonly form chains up to 200 μ long. Individual cells in a trichome may separate

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* Pringsheim (Bacteriol. Revs., *21*, 1957, 69) reports a prominent sheath exists but cannot be demonstrated by means of nigrosin. It stains pink with methylene blue whereas the cells stain a dark indigo.

as discoid elements and may grow out into trichomes; common in peat and cow dung
Caryophanon p. 127
M p. 831

General morphology similar to that found in *Caryophanon*, but individual trichomes are shorter and usually composed of four cells or four cell pairs; trichomes are chain-forming but *nonmotile;* recorded from the oral cavity of man and domestic animals
Simonsiella p. 128
M p. 833

Not as above...................... **15**

15. Long trichomes attached by a globular holdfast to the intestinal walls of some insects and millipedes; a single endospore may be produced in any or all cells of the trichome, and if so, it usually lies in an oblique position....**Arthromitus** p. 128
M p. 835

Trichomes 1.5 to 22.0 μ long; found in the cytoplasm of the rhizopod *Pelomyxa palustris*, generally aggregated close to the nucleus......................**Cladothrix**
M p. 930

Not as above...................... **16**

16. Entire trichomes unattached and actively motile by a gliding action on a solid surface; no flagella..................**17**

Trichomes attached or free; nonmotile; abstricted cells may be motile with a gliding action on solid surfaces............. **19**

Note: References have been made in the literature to the multicellular nature of many small cells previously regarded as unicellular. They are mostly cells not more than 6 microns long and they stain uniformly or more frequently show a granulated or barred staining with simple stains but show a multicellular character when special techniques are applied to show cell walls. They will be found by other characteristics to belong to genera located in Section F *et seq*

17. Organisms oxidize hydrogen sulfide, depositing sulfur inside the cell
Beggiatoa p. 129
M p. 838

Not as above...................... **18**

18. Trichomes composed of cells which
not clearly articulated at the juncti
complete trichomes very flexible
Vitreoscilla p.
M p.

Trichomes composed of rod-like elem
with little individual flexibility; ben
occurs freely at the junctions
Bactoscilla p.
M p.

19. Colorless trichomes; attached; cells
abstricted from the terminal position
are motile by a gliding action on a s
surface. Sulfur is not deposited internal
Leucothrix p.
M p.

Colorless trichomes; arranged in bund
nonmotile; each cell in the trichome c
tains one or more gas vacuoles which gle
reddish in transmitted light
Peloploca p.
M p.

Cells deposit sulfur internally
Thiothrix p.
M p.

Note: Thiothrix is inserted here althou
classical descriptions of the genus indic
a sheath.

SECTION F

1. Pear-shaped cells; 1.0 to 2.0 by 4.0
5.0 μ; nonmotile; grow attached to ea
other or to solid surfaces by a holdf
secreted from the narrow end; sessile; m
tiply by longitudinal fission and by buddi
at the free end.......**Pasteuria** p.
M p.

Not as above.........................

2. Curved or straight rods which produ
a well differentiated stalk by which th
may attach to a surface; a single cell occu
at the end of each stalk except during t
process of multiplication.............

Not as above.........................

3. Cells curved; stalk produced as a co
tinuation of the cell wall parallel to the lo
axis of the cell; reproduction by transve
fission of the cell from the free end, wi

daughter cell developing a single polar gellum and then breaking away and procing a stalk from the end of the cell at ich the flagellum is located
Caulobacter p. 82
M p. 213

ot as above; cells produce a stalk at right gles to the main axis of the cell...... 4

Large rods, 2.0 by 6.0 to 12.0 μ, borne the ends of lobose, dichotomously anched stalks composed of gum, forming gummy colony which floats on water; y become attached....**Nevskia** p. 84
M p. 216

ot as above; stalks composed of or imegnated with ferric hydroxide; dissolve mpletely in hydrochloric acid; cells rved with the stalk secreted from the ncave side; reported only from iron-bearg waters........................ 5

Stalks ribbon-like and usually twisted; lls located terminally
Gallionella p. 83
M p. 214

(See Note on Gallionella, p. 83.)

alks horn-shaped; not twisted; round in oss section.......**Siderophacus** p. 83
M p. 216

Obligate autotrophs which oxidize ferus to ferric iron at low pH in mineral nthetic media; Gram-negative rods... 7

ot as above...................... 8

Thiosulfate is oxidized
Thiobacillus p. 60
(*T. ferrooxidans*) *M p. 83*

hiosulfate is not oxidized
Ferrobacillus p. 70
(*F. ferrooxidans*) *M p. 227*

Organisms which store oxides of mannese or iron either in the cell membrane, the cell wall, or in the surrounding caples; found in water and mud........ 9

Note: In the absence of further informaon, these organisms are identified on the sis of their iron-depositing characteriscs. Most iron organisms studied in pure lture metabolize the organic compound hich forms the iron chelate, and the berated iron then chelates with some cell component. Citrate-utilizing organisms will, for example, release iron from ferric ammonium citrate. Accumulation of the iron in or on the cell may depend only on the nature of the cell substance. Pure culture studies may place these organisms in more commonly recognized genera. They should also be treated as non-iron-depositing cells and should be followed through the key.

Not as above...................... **13**

9. Cells encapsulated or embedded in mucus or surrounded by a torus of iron... **10**

Not as above; iron or manganese stored in cell membrane or cell wall
Siderobacter p. 69
M p. 226

10. Encapsulated cells occurring singly or in short chains, each capsule being completely surrounded by a ring (torus) heavily impregnated with iron or manganese giving the general appearance of links in a chain
Naumanniella p. 69
M p. 223

Not as above...................... **11**

11. Cells surrounded by a ring (torus) which is open at one end; cells motile by means of two unequal polar flagella
Ochrobium p. 69
M p. 225

Note: The unequal lengths of the flagella suggest that this may be an algal or protozoan cell.

Not as above...................... **12**

12. Rods arranged at random in zoogloea; encrusted with iron
Sideromonas (Including *Siderocapsa major*.......................... p. 68
M p. 222

Cells arranged in pairs or chains in capsules...........**Ferribacterium** p. 68
M p. 221

13. Cells *in mass* appear green to greenish yellow; contain a photosynthetic pigment which is not bacteriochlorophyll or chlorophyll "a" but which may be chlorobium chlorophyll; individual cells usually colorless; grow anaerobically when exposed to light, oxidizing sulfide to sulfur, which is deposited outside the cell; no growth aerobically.......................... **14**

Not as above.......................... **18**

14. Cells found adherent to the surface of other organisms, apparently living in symbiosis with them................ **15**

Note: The taxonomic significance of these groups is doubtful, but until they are isolated from the supposed symbiont and studied separately, they must be treated in this fashion.

Cells free-living..................... **17**

15. Green cells attached to the surface of a protozoan cell

Chlorobacterium p. 53
M p. 65

Green cells attached to bacteria....... **16**

16. Aggregates small, barrel-shaped; actively motile, consisting of a central, polar flagellated, rod-shaped cell covered with the green organisms; green cells 0.5 to 1.0 by 1.0 to 2.5 μ, usually 8 to 16 surrounding the central cell; aggregates measure 2.5 to 5.0 by 7 to 12 μ

Chlorochromatium p. 53
M p. 65

Aggregates large, long, and cylindrical; nonmotile; consist of green cells, 0.5 to 1.0 by 2.0 to 4.0 μ, lying on the surface of a slime capsule which covers the inner cylindrical cell. They are themselves covered by a layer of slime. Aggregates measure 7.0 to 8.0 μ wide by up to 50 μ long

Cylindrogloea p. 53
M p. 66

17. Encapsulated cells forming characteristic aggregates consisting of net-like structures, irregular three-dimensional or two-dimensional masses in which cells lie in parallel strands.....**Pelodictyon** p. 53
M p. 63

Cells may produce slime but usually remain dispersed; in young healthy cultures, small ovoid rods, 0.7 to 0.9 by 1.5 μ, forming chains; at pH 7.5 to 8.0, involution forms, such as cork-screw type rods and cocci, are characteristic....**Chlorobium** p. 52
M p. 62

18. Cell masses are various shades of red
or purple..........................
Not as above.......................
19. Cells contain bacteriochlorophyll
carotenoid pigments; capable of gro
anaerobically when exposed to light;
ceed to................Section J p
Not as above.......................
20. Cells motile only by a gliding m
ment on solid surfaces or along each ot
single cells nonmotile when free in solut
Motility can be observed at the glass-w
interface on a slide or on the surfac
agar plates where cells move singly o
groups, frequently leaving a trail of sl
behind. Cells are flexible, the extent of
flexibility being dependent on length
turgidity (see note below)............

Note: This subdivision is represented
two main groups. In one the cells are u
ally less than 10 μ long, frequently c
2.0 to 5.0 μ; Gram-negative; in wet pr
arations fixed with osmic acid, they m
appear as blunt-ended rods; in stai
preparations subjected to heat fixati
they are frequently curved, S-shaped, b
or spirilliform. In the other, the cells
usually longer than 10 μ and may fo
articulated chains.

There are, however, much shorter rep
sentatives of this subdivision which,
cause of their shortness, are more ri
and resemble ordinary bacteria in ma
respects except for the gliding movem
and lack of flagella. Species of both grou
so far described have been found in fr
and salt water and in soil and decompos
organic matter, especially dung.

Not as above; cells nonmotile or mot
when free in solution, by means of flage
proceed to............Section G p.
21. Cells which, on rabbit dung or bacter
cell agar or other suitable media, produ
spherical or rod-shaped microcysts eit
lying free among the rods or borne
macroscopically visible fruiting bod
formed by transformation of whole or p
of the population of rod-shaped cells; ce
rarely more than 10 μ long; proceed to
Section L p.

t as above...................... 22

Rod-shaped cells forming articulated
ins.............**Bactoscilla** p. 130
M p. 848

t as above...................... 23

Filaments from 12 to 100 μ or more in
gth, highly flexible and actively motile
gliding motion; cells may bend and wave
; do not rotate..**Microscilla** p. 130
M p. 849

t as above...................... 24

Cells usually less than 12 μ long. Two
ups are possibly represented here. If the
onies are pigmented yellow, pink, green,
nge, or black, they probably belong to
genus.........**Cytophaga** p. 132
M p. 858

they are white and albuminous, they
y belong to Soriano's genus
Flexibacter*
M p. 858

SECTION G

Organisms will not grow on meat ex-
ict or other complex organic media;
ict autotrophs which use carbon dioxide
the sole source of carbon and which ob-
n their energy from the oxidation of car-
n monoxide or other inorganic substances
2

Vote: The genus *Methanomonas* has to be
isidered here. The information in the
inual does not indicate whether the or-
iism is a strict or facultative autotroph.
vorkin and Foster (J. Bacteriol., *72*(5),
56, 646) describe an organism under the
me of *Pseudomonas methanica* which
ey considered to be identical with *Meth-*
omonas methanica (Sohngen) Orla-Jen-
n. Methane and methanol were the only
rbon compounds of those tested which
oved satisfactory for growth. Foster† states that the organic growth factors described as necessary in the original paper are not necessary and that the organism grows well in a completely inorganic medium (see section on media) in the presence of methane. Polar flagellates should be tested under these conditions for their ability to oxidize methane.

Not as above........................ 6

2. Organisms oxidize ammonia to nitrite
3

Organisms oxidize nitrite to nitrate.... 5

Organisms oxidize inorganic sulfur compounds............**Thiobacillus** p. 60
M p. 83

Organisms oxidize ferrous iron
Ferrobacillus p. 70
M p. 227

Organisms oxidize carbon monoxide
Carboxydomonas p. 58
M p. 77

Note: It is reasonably certain that *Carboxydomonas* is also heterotrophic and may be identical with *Hydrogenomonas.*

3. Cells encapsulated; form zoogloea... 4

Cells not encapsulated
Nitrosomonas p. 56
M p. 68

4. Zoogloea encysted
Nitrosocystis p. 56
M p. 70

Zoogloea not encysted
Nitrosogloea p. 57
M p. 71

5. Zoogloea formed...**Nitrocystis** p. 57
M p. 73

Zoogloea not formed
Nitrobacter p. 57
M p. 72

6. Gram-positive................... 7

Gram-negative; proceed to
Section H p. 26

7. Aerobic........................ 8

Anaerobic........................ 35

8. Endospores formed..**Bacillus** p. 115
M p. 613

Note: Five species of *Clostridium* (entries 89 to 93 in the *Manual*) are described as aerotolerant. Their growth under aerobic conditions is very restricted.

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* Not recognized in the *Manual.* See Sor-
io, Rev. Arg. Agron., *12,* 1945, 120 and An-
iie van Leeuwenhoek., J. Microbiol. Serol.,
1947, 215.

† Personal communication, October 10,
7.

No endospores produced 9

9. Organisms show distinct branching in young cultures 10

Organisms do not branch 19

Note: No provision appears to be made for organisms which, under optimal growth conditions, produce long, *unbranched* filaments which, like *Nocardia,* eventually disintegrate into a series of short rods. The *Manual* descriptions of *Nocardia globerula* and *N. rubropertincta* suggest such forms. The author has observed others in rabbit dung media. It would seem better to assign such forms to a new genus in order to retain the branching character in *Nocardia.*

10. Branching mycelium produced in young cultures 11

Branching very rudimentary, limited to simple branching of isolated rods, no mycelium formed 16

11. Long, branching filaments which show little or no tendency to fragment completely into short bacillary and coccal elements 12

Organisms show dichotomous branching in young cultures. Filaments later divide into unicellular, rod-shaped segments which become arranged in zigzag fashion. Subsequently a narrow tube is produced from one end of each rod and grows out, finally developing to the same width as the original rod. Filaments may also reproduce by breaking down into spore-like elements reminiscent of the sporogenous hyphae of *Streptomyces.* L-forms common. Found in the mouth . . . . **Leptotrichia** (*L. dentium*)

(This genus is not listed in the *Manual.* See Baird-Parker and Davis, J. Gen. Microbiol., *19,* 1958, 446.)

Organisms produce a well defined mycelium in the early stages of development and then completely fragment into short bacillary elements. When aerial mycelium is produced it also fragments into rods and coccal
elements but does not produce differe
ated conidia. Fragmentation of the m
lium may commence within a few hour
may be delayed for several days
Nocardia p.
M p.

Note: a. Cultures of *Actinoplanes,* w
cultured on certain media, fail to prod
sporangia and resemble *Nocardia.*

b. *Actinomyces,* generally regarded
anaerobic, is considered by some to
microaerophilic. Organisms growing po
aerobically but better anaerobically sho
be compared carefully with the *Actinom*
spp. According to Bisset and Moore
Gen. Microbiol., *3,* 1949, 387) compon
rods of *Nocardia* are each composed o
number of very short cells, *i.e.* they
multicellular, whereas component rods
Actinomyces are unicellular. They also n
that branches occurring in *Nocardia*
Actinomyces are separated by a septum
the point of origin whereas in *Streptomy*
the branch arises from the component
without the development of a septu
Since a limited number of species was
amined, these claims require further s
stantiation.

c. Gordon (J. Bacteriol., *75,* 1958, 2
claims that aerial chains of spores are p
duced by *Nocardia asteroides* and that th
are indistinguishable morphologically fr
those of *Streptomyces.* This claim should
examined in the light of Bisset and Moor
(Note b) claim for multicellularity of
Nocardia with the view to transferring
species to *Streptomyces.*

12. Organisms which grow over or wit
submerged plant tissues and on cert
laboratory media, producing conidia wit
sporangia borne on aerial hyphae. . . . .
Not as above . . . .

13. Mycelium penetrates the submerg
plant tissue; conidia, 1.0 to 1.5 μ in dia
eter, produced in coils or irregularly wit
the sporangia; conidia globose to sligh
angular; sporangia, 8.4 to 22.0 μ in dia
eter, borne on long aerial hyphae; coni

ile; germinate to produce a branched
celium.........**Actinoplanes** p. 127
M p. 826

celium grows over the surface of sub-
rged plant tissue; conidia spherical, 1.8
2.0 μ in diameter, produced in coils
hin the sporangia; sporangia 7 to 19 μ in
meter produced apically or on branches
aerial hyphae; conidia *nonmotile* and
cibly ejected from a protuberance
med from the sporangium wall when the
rangia are immersed in water
Streptosporangium p. 127
M p. 828

. Chains of conidia produced from the
ls of aerial hyphae
Streptomyces p. 121
M p. 744

Note: a. Cultures of *Actinoplanes* or
eptosporangium on some laboratory me-
a are morphologically similar to *Strepto-
ices.*

b. Certain species of *Nocardia* in which
gmentation is delayed may produce
ickened aerial hyphae which abstrict cells
m the tip, proceeding towards the base
d finally involving the whole mycelium.
rly stages in this change may be con-
sed with *Streptomyces, e.g. N. fordii* and
paraffinae.

c. See also Notes b and c under G, **11.**

nidia produced in pairs either sessile on
mycelium or on a short conidiophore,
e pair of conidia arranged so that only
e of the pair is in contact with the myce-
m or conidiophore
Waksmania p. 123

Note: This genus does not appear in the
anual. See Lechevalier and Lechevalier
. Gen. Microbiol., *17*, 1957, 104).

nidia produced singly on the tips of
ort side branches; branches may occur
gly or in clusters giving the appearance
bunches of grapes................ **15**

ycelium persistent with no production of
nidia. These forms should be regarded in
e same sense as the *Mycelia sterilia* of the fungi until something more definite is known of possible fruiting structures.

15. Do not grow between 50° and 60° C
Micromonospora p. 126
M p. 822

Growth occurs between 50° and 60° C.; aerial mycelium produced
Thermoactinomyces p. 126
M p. 824

16. Acid-fast...**Mycobacterium** p. 118
M p. 695

Nonacid-fast...................... **17**

17. Rudimentary branching rods only under aerobic conditions or in acid media anaerobically; produce chains of cocci or short rods in neutral media under anaerobic conditions; *propionic acid produced from lactic acid*...**Propionibacterium** p. 110
M p. 569

Cells have a definite cyclic development; spherical cells germinate at one or more points to produce rod-shaped cells which elongate and divide. At the point of division, growth of the cells continues at an angle to the original axis. When side branches are equal in size to the parent, cell division occurs at the angle. This process is repeated during the growth of the colony. *In older colonies the rods transform entirely into a mass of cocci.* Rods are most frequently Gram-negative with Gram-positive granules; the coccal forms are frequently Gram-positive. Recorded mainly from soils........**Arthrobacter** p. 114
M p. 605

Note: The emphasis lies on the final transformation into cocci. Some authorities may consider that limited true branching may occur. If this is admitted, the dividing line between *Arthrobacter* and *Nocardia* becomes very slim. The author's observations of *Arthrobacter globiformis* fit the above statement, and it is suggested that these criteria be adopted, with true branching forms which later disintegrate being assigned to *Nocardia.*

Organisms produce both coccoid and rod-shaped forms, the latter frequently branch-

ing. Coccoid resting cells are produced which may germinate at one or more points. The coccoid forms generally predominate.......... **Mycococcus** p. 119
M p. 707

Note: The descriptions given for species in this genus are like early descriptions given for *Arthrobacter* and in some instances could fit *Nocardia*.

Not as above........................ **18**

18. Organisms disintegrate filter paper in 0.5 per cent peptone water; produce clearing on precipitated cellulose agar plates; motile (except *C. flavigena*)
Cellulomonas p. 114
M p. 601

Not as above
possibly **Corynebacterium** p. 111
M p. 579
or **Jensenia** p. 121

Note: Bisset and Moore (J. Gen. Microbiol., *3*, 1949, 387) describe a new genus, *Jensenia*, to include the "soil diphtheroids." According to these authors rod-shaped elements of the true *Corynebacterium* are multicellular and do not branch whereas those of the majority of "soil diphtheroids" are unicellular with the branches separated from the parent cell at the point of origin by a septum.

19. Motile at 37° or at 25° C......... **20**
Nonmotile........................ **27**

20. Organisms pathogenic to warm-blooded animals causing monocytosis; catalase-positive; acid produced from glucose, salicin, and esculin
Listeria p. 112
M p. 597
Erysipelothrix (T and W, 4th ed.)

Not as above........................ **21**

21. Pathogenic to plants............. **22**
Not pathogenic or not known to be pathogenic to plants.................... **24**

22. Yellow colonies; polar flagella
possibly **Corynebacterium** p. 111
(*C. flaccumfaciens* or *C. tritici*) *M p. 579*
Or............. **Xanthomonas** p. 62
(*X. proteamaculans* or *X. conjac*)
M p. 152

Yellow colonies; peritrichous flagella
Erwinia p.
(*E. citrimaculans*) *M p.*
Bacterium (T and W, 4th e
Colonies not yellow.................

23. Flagella polar
Corynebacterium p.
(*C. poinsettiae* or *C. hypertrophicans*)
M p.
Or.............. **Pseudomonas** p.
(*P. polygoni*) *M p.*
Flagella peritrichous .. **Erwinia** p.
(*E. carnegeana*) *M p.*
Bacterium (T and W, 4th e

Note: Erwinia species are generally garded as Gram-negative. Gram-varia species are recorded.

24. Organisms disintegrate filter paper 0.5 per cent peptone water; produce cle ing on precipitated cellulose agar plates
Cellulomonas p. 1
M p. 6
Not as above.......................

25. No acid from carbohydrates......
Acid from carbohydrates
Brevibacterium p. 1
M p. 4

(Including *Flavobacterium suavolens* a *F. marinum.*)

Note: a. Bolcato (Antonie van Leeuwe hoek., J. Microbiol. Serol., *23*, 1957, 35 has described a new organism, *Microbo terium mobile*, which has most of the cha acteristics assigned to *Microbacterium* at **29** except that the cells occur singly and chains and are motile with peritricho flagella: lactic acid produced is mainly o tically inactive.

b. Harrison and Hansen (J. Bacteric *59*, 1950, 444–445) also record a new varie of *Lactobacillus plantarum* (var. *mobili* which is motile with peritrichous flagel and produces dextrorotatory lactic acid.

26. Organisms occur in long chains; col nies colorless and spreading
Kurthia p. 1
M p. 5

ganisms arranged singly; colonies yellow
Brevibacterium p. 103
(*B. sulfureum*) *M p. 490*

Acid-fast... **Mycobacterium** p. 118
M p. 695

t acid-fast........................ **28**

Organisms 0.2 to 0.4 by 0.5 to 2.5 μ;
g filaments common in rough colonies;
ur singly and also in chains; pinpoint
nsparent colonies on agar in 24 hours
37° C., extending on further incubation
1.5 mm.; acid only produced from glu-
e and lactose and some other carbohy-
ates but not from esculin; final pH in
ucose broth, approximately 6.0; hydrogen
lfide is produced; causes swine erysipelas,
man erysipeloid, mouse septicemia and
ections in sheep, birds, and fish
Erysipelothrix p. 113
p. 599

ot as above........................ **29**

. Rods 0.4 to 0.7 by 1 to 3 μ; show granu-
r staining with methylene blue; arranged
angular fashion similar to the coryne-
cteria; only two species recorded, both
which produce acid from glucose, fruc-
se, and mannose; catalase-positive; resist
ating to 72° C. for 15 minutes; normally
und in dairy products and equipment
Microbacterium p. 113
M p. 600

ot as above........................ **30**

. Organisms grow on agar under aerobic
nditions only if heavy inocula are used;
ghtly pleomorphic, including branched
rms; *propionic acid is produced from glu-*
se and also from lactic acid; catalase-posi-
ve....... **Propionibacterium** p. 110
(*P. arabinosum*) *M p. 569*

ot as above........................ **31**

1. Small spindle-shaped cells usually oc-
urring in long intertwined chains in liquid
edia; microaerophilic; ferments glucose
ith the production of ethyl alcohol with
nall amounts of CO_2 and acetic acid and
ossibly lactic and formic acids; acid and
as in glucose in 3 to 5 days
Zymobacterium p. 111
M p. 577

Not as above........................ **32**

32. Colonies usually 1 mm. or less in diameter, colorless, little or no growth on media devoid of carbohydrates; rods frequently arranged in chains, grow in glucose broth, producing a pH much below 6.0; acid produced from lactose by all species except *L. delbrueckii* and *L. brevis*
Lactobacillus p. 107
M p. 543

Not as above........................ **33**

33. Lactose fermented; cells pleomorphic; arranged in palisades and Chinese letter forms; frequently bar-shaped, beaded and clubbed..... **Corynebacterium** p. 111
p. 579

Note: Bisset (J. Gen. Microbiol., *3*, 1949, 93) states that the apparent barred and beaded staining is due to the fact that the so-called rods of corynebacteria are multicellular.

Lactose not fermented............... **34**

34. Organisms occur singly, in pairs or in short chains; not pleomorphic
Brevibacterium p. 103
M p. 490

(See also *B. healii.*)

Organisms normally found in palisade or Chinese letter forms, pleomorphic; barred, and beaded, and club-shaped forms common........ **Corynebacterium** p. 111
M p. 579

Note: Criteria for separation of these two genera are inadequate. Multicellularity in *Corynebacterium* may be the decisive factor (see note to G, **33**).

35. Organisms produce a true branching mycelium which later disintegrates into simple rods, cocci, and rods with remnants of branches....... **Actinomyces** p. 120
M p. 742

Organisms produce a branching mycelium which does not readily disintegrate; conidia produced singly on short condiophores
Micromonospora p. 126

Note: The *Manual* does not list any anaerobic species of *Micromonospora.* Hungate (J. Bacteriol., *51*, 1946, 51) describes an anaerobic cellulose-digesting organism belonging to this genus.

Note: Bisset (J. Gen. Microbiol., *17*, 1957, 562) describes a monosporous actinomycete.

Organisms do not branch, or if branching occurs, it is limited to bifurcations and branching of isolated rods; no mycelium formed **36**

36. Endospores produced **37**

No endospores produced **38**

37. Organisms reduce CO_2 to CH_4 while oxidizing secondary alcohols to ketones and primary alcohols to acids

Methanobacterium p. 116
(*M. omelianskii*) *M p. 250*

Not as above. . . . **Clostridium** p. 116
M p. 634

38. Cells motile; gas produced from peptone in the absence of carbohydrates

Cillobacterium p. 109
M p. 566

Note: Glucose and other carbohydrates are fermented by all species; butyric acid is frequently among the byproducts.

Cells nonmotile **39**

Smith and Hungate (J. Bacteriol., *75*, 1958, 713) describe an organism which produces methane from hydrogen and formate which they call *Methanobacterium ruminantium.* Data given do not permit comparison beyond this point.

39. Visible gas produced in culture media either in the presence or absence of carbohydrates **40**

Note: In the ensuing Sections **40** to **44** inclusive, the phrase "acid from" infers "acid" or "acid and gas," since it is not clear whether gas produced in the presence of carbohydrate arises from the fermentation of the sugar or action on the peptone.

Visible gas in culture media either in the presence or absence of carbohydrates; glucose fermentation not recorded; pathogenic to guinea pigs; rods occur singly, in pairs, in V-formation, in short chains, and in clumps. (See *Eubacterium niosii* and *Eubacterium quintum.*)

No visible gas produced in culture m
in the presence or absence of carbo
drates . . . .

40. Acid produced from glucose, lact
and fructose . . . .

Acid produced from glucose and fruc
but not from lactose . . . .

Acid produced from glucose and lactose
not from fructose . . . .

Acid produced from glucose but not fr
lactose or fructose . . . .

41. Large rods with rounded ends occ
ring as short swollen and long cur
forms; bifurcation common; acid produ
also from maltose, galactose, and sucro
strict anaerobes

Catenabacterium p. 1
(*C. filamentosum*) *M p. 5*

Slender rods sometimes undulating a
filamentous; form acute V- and Y-shap
angles giving an appearance of false bran
ing; acid also from galactose and trehalo
strict anaerobes **Ramibacterium** p. 1
(*R. pseudoramosum*) *M p. 5*

Microaerophilic, pleomorphic organisms
curring as chains of cocci in acid med
anaerobically and as rods with branchi
forms aerobically; aerobic growth ve
poor; propionic acid produced from lac
acid; limited gas from carbohydrates b
none from peptone

Propionibacterium p. 1
M p. 5

Small, spindle-shaped cells usually occu
ring in long intertwined chains in liqu
media; ferments glucose with the produ
tion of ethyl alcohol and small amounts
CO_2 and acetic and possibly lactic an
formic acids; *no butyric or propionic ac
produced*; acid and gas from glucose in 3
5 days

Zymobacterium p. 1
(*Z. oroticum*) *M p. 5*

Rods occurring singly, in pairs, or in sho
or long chains; strict anaerobes

Eubacterium p. 1
M p. 5

(See also *Lactobacillus brevis* and *Cory
bacterium avidum.*)

Organisms occur in long chains; acid n glucose, fructose, maltose, sucrose, ctose, xylose, and arabinose; propionic produced among the byproducts; strict erobes.... **Catenabacterium** p. 109
(*C. contortum*) *M p. 560*

anisms arranged in chains and in acute and Y-shaped forms suggestive of false nches; gelatin not liquefied; nitrites not duced from nitrates; no acid from mal- e; butyric acid is produced; strictly erobic..... **Ramibacterium** p. 109
dentium and *R. alactolyticum*) *M p. 563*

roaerophilic pleomorphic organisms oc- ring as chains of cocci in acid media erobically and as rods with branching ms aerobically; aerobic growth very r; propionic acid produced from lactic d; limited gas from carbohydrates but ne from peptone
Propionibacterium p. 110
M p. 569

her than above; organisms occur singly in short chains; acid from maltose; tyric acid is produced by one species; ict anaerobes... **Eubacterium** p. 108
M p. 552
(*E. foedans*, *E. quartum*, *E. limosum*)
(See also *Lactobacillus brevis* and *Coryne- acterium avidum.*)

. Organisms occur in short or long chains th zigzag arrangement and acute V- and orms suggestive of false branching; pionic acid produced by one species
Ramibacterium p. 109
. *ramosum* and *R. ramosoides*) *M p. 563*

ganisms occur singly or in a long, sinuous ain of cells....**Eubacterium** p. 108
(*E. rectale*) *M p. 552*

Note: There is a strong resemblance be- een *Ramibacterium ramosoides* and *Eu- cterium rectale.*

. Organisms occur in short chains and as ng pleomorphic filaments; acid produced om glucose, maltose, sucrose, galactose, d glycerol; hydrogen sulfide is produced; latin is liquefied; butyric acid is produced
Catenabacterium p. 109
(*C. helminthoides*) *M p. 560*

Organisms arranged singly; butyric acid, acetic acid and CO_2 produced from glucose, maltose, and lactic acid
Butyribacterium p. 111
(*B. rettgeri*) *M p. 577*

Organisms arranged singly; amines, ammonia, and nitrogen produced
Eubacterium p. 108
(*E. obstii*) *M p. 552*

Organisms arranged predominantly in Y-shaped forms suggestive of false branching; acid from glucose and galactose; gelatin not liquefied...... **Ramibacterium** p. 109
(*R. pleuriticum*) *M p. 563*
(See also *Cornyebacterium diphtheroides.*)

45. No acid from carbohydrates
Eubacterium p. 108
M p. 552

Acid from carbohydrates............. **46**

46. Acid from glucose, lactose, and fructose............................ **49**

Acid from glucose and lactose but not from fructose..... **Catenabacterium** p. 109
(*C. lottii*) *M p. 560*
(See also *Lactobacillus caucasicus.*)

Acid from glucose and fructose but not from lactose........................... **47**

Acid from glucose but not from lactose or fructose.......................... **53**

47. Organisms pathogenic to mice and rabbits; cause urinary tract infection in horses, cattle, sheep, and dogs; propionic acid not produced from lactic acid; strictly anaerobic.... **Corynebacterium** p. 111
(*C. renale*) *M p. 579*

Not as above..................... **48**

48. Organisms found mainly in fermenting foods; *propionic acid produced from lactic acid*; microaerophilic to anaerobic
Propionibacterium p. 110
M p. 569

No propionic acid produced from lactic acid; microaerophilic to anaerobic
Lactobacillus p. 107
M p. 542

Note: Hayward, Hale, and Bisset (J. Gen. Microbiol., *13*, 1955, 292) suggest that *Lactobacillus bifidus* should be placed in the genus *Bifidobacterium* Orla-Jensen, 1924.

See also *Leptotrichia buccalis* (Hamilton and Zahler, J. Bacteriol., *73*, 1957, 368).

49. Butyric acid produced........... **50**
Propionic acid produced............. **51**
No propionic or butyric acid produced. **52**

50. Organisms occurring in very long chains
Catenabacterium p. 109
(*C. catenaforme*) *M p. 560*
Organisms occurring singly, in pairs and in clumps.......... **Eubacterium** p. 108
(*E. parvum*) *M p. 552*
(See also *Corynebacterium avidum.*)

51. Strictly anaerobic
Eubacterium p. 108
(*E. disciformans*) *M p. 552*
Microaerophilic to anaerobic; propionic acid produced from lactic acid; catalase-positive.... **Propionibacterium** p. 110
M p. 569

52. Long rods, 0.3 by 8.0 μ, with tapered, rounded, or swollen ends; often occurring in short or long chains
Catenabacterium p. 109
(*C. leptotrichoides*) *M p. 560*
Slender rods, 0.5 to 0.7 by 2 to 8 μ, occurring in short or long chains; bifurcations common; produce lactic and volatile acids from glucose; microaerophilic to anaerobic
Lactobacillus p. 107
(*L. bifidus*) *M p. 542*
Rods 1.0 by 3.0 μ; produced in short or long chains; acetic and lactic acids produced from glucose
Eubacterium p. 108
M p. 552

Note: E. crispatum has a similar sugar fermentation range to that of *Lactobacillus bifidus*, but acetic acid appears to predominate.

See also *Leptotrichia buccalis* (Hamilton and Zahler, J. Bacteriol, *73*, 1957, 386).

53. Propionic acid produced.......... **55**
Butyric acid produced from lactic acid; acid from glucose and maltose
Butyribacterium p. 111
(*B. rettgeri*) *M* p. 577
No propionic or butyric acid produced.

54. Organisms arranged in short to v
long chains... **Catenabacterium** p.
(*C. nigrum*) *M p.*
Organisms occurring singly and in gro
arranged in a palisade or Chinese let
fashion...... **Corynebacterium** p.
(*C. granulosum and C. parvum*) *M p.*

55. Catalase-positive; propionic acid p
duced from lactic acid
Propionibacterium p. 1
M p. 5
Catalase-positive; propionic acid produ
from glucose but not from lactic acid
Corynebacterium p. 1
(*C. acne*) *M p. 5*

SECTION H

1. Aerobic.........................
Anaerobic; proceed to... Section I p.

2. Cells 1.4 to 1.6 μ wide and 10 to 50
long, forming chains usually over 1
microns in length; individual rods a
characterized by constrictions which lat
developed into cross walls followed b
division; cells may branch, the branch a
pearing at or near the site of constricti
but not visibly connected with the ma
axis; motile by means of peritricho
flagella; form small, bluish white, iridesce
colonies on peptone yeast extract aga
particularly with added acetate
Lineola p. 1
M p. 8
Not as above.......................

3. Organisms isolated from cases of gra
uloma inguinale; grow only in the yolk s
of the developing chick embryo or in co
densation water of a sloped medium pr
pared by adding 50 per cent unheated e
bryonic yolk to melted and cooled nutrie
agar..... **Calymmatobacterium** p. 7
M p. 4
Donovania (T and W, 4th ed
Pleomorphic organisms parasitic on
within erythrocytes of man and other ve
tebrates, causing infections of lower a
mals; stain by Giemsa's stain witho

le differentiation into nucleus and plasm; grown with variable success in isolid agar containing whole blood. ceed to .... Section K p. 45

anisms grow in glucose-blood-bouillon r as Gram-negative rods 1.0 μ in length; r in masses as coccoid and ellipsoidal s 0.3 to 0.5 μ in diameter, extracellu- y lining the intestinal epithelium of the ep ked, *Melophagus ovinus*

Wolbachia p. 139
(*W. melophagi*) *M p. 953*

t as above 4

Organisms isolated from nodules on the ts of leguminous plants and capable of ducing nodules on the host plant

Rhizobium p. 85
M p. 285

t as above 5

Organisms capable of continued growth a glucose, sucrose, or mannitol mineral ts medium devoid of nitrogen compounds 6

t as above 7

Motile with polar flagella

Azotomonas p. 64
M p. 198

otile with peritrichous flagella or non- otile:

6a. Straight or slightly curved or irregular, locally swollen or buckled rods characterized by the presence at the extremities of highly refractile spherical bodies, presumably consisting of lipoids; grow in acid media at pH 4.5

Azotobacter (7th ed.) p. 84
M p. 283
Beijerinckia Derx 1950

6b. Spherical to oval yeast-like cells exceeding 2 μ in width which do not form cysts (arthrospores). Do not produce acid from carbohydrates or grow at pH 4.5.......**Azotobacter** (7th ed.) p. 84
M p. 283
Azotococcus Tchan 1953
(See Tchan, Proc. Linnean Soc. N. S. Wales, *78*, 1953, 85)

6c. Rod-shaped to spherical cells 2 μ or more in width which produce cysts (arthrospores). Do not produce acid from carbohydrates or grow at pH 4.5

Azotobacter (7th ed.) p. 84
M p. 283

Not as above 7

Note: Nitrogen fixation has also been recorded for *Aerobacter aerogenes* and some other organisms which will key out in a subsequent section.

7. Endospores produced

Bacillus p. 115
M p. 613

Note: Most species of the genus *Bacillus* are Gram-positive.

Endospores not produced **8**

8. Organisms grow well on yeast water medium containing up to 10 per cent alcohol, with oxidation of the latter to acetic acid; growth very poor or absent on media containing no carbohydrates or alcohols.

a. Polar flagella; do not oxidize acetate

Acetomonas Leifson* 1954
Acetobacter (7th ed.) p. 62
M p. 183

b. Peritrichous flagella or nonmotile; oxidize acetate to CO_2 and H_2O

Acetobacter Leifson* 1954
Acetobacter (7th ed.) p. 62
M p. 183

Note: Erwinia carotovora is recorded to be able to oxidize 5 per cent ethyl alcohol.

Not as above **9**

9. Organisms grow poorly on peptone media in the absence of carbohydrates; good growth of white, raised, round colonies 1 mm. in diameter on wort agar under anaerobic conditions; only slight growth, if any, aerobically in the presence of sugars; glucose fermented with the production of carbon dioxide, lactic acid, and up to 10 per cent ethyl alcohol; motile by means of a single polar flagellum

Zymomonas p. 65
M p. 199

Organisms which will not grow on common laboratory media; grow in sucrose broth containing less than 0.5 per cent NaCl and

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* See Notes on Acetobacter, p. 62.

a very low concentration of meat extract; will grow in selected synthetic media with the use of pneumococcal capsular polysaccharide as the sole source of carbon
Saccharobacterium

Note: This genus is recorded in the sixth but not in the seventh edition of the *Manual.*

Not as above........................ **10**

10. Colonies with a purple pigment
Chromobacterium p. 86
M p. 292

Note: Chromobacterium marismortui has a bluish brown pigment; see also *Pseudomonas iodinum* and *Pseudomonas beijerinckii.*

Colonies red at 37° or 25° C., sometimes with a red, water-soluble pigment.... **11**
Other than above.................. **17**

11. Red color due to prodigiosin; motile by means of peritrichous flagella or nonmotile................ **Serratia** p. 94
M p. 359
Chromobacterium (T and W, 4th ed.)
Red color not due to prodigiosin...... **12**

12. Obligate halophiles requiring 20 to 30 per cent salt for growth; highly pleomorphic
Halobacterium p. 66
M p. 207
Not obligately halophilic............. **13**

13. Organisms metabolize alkylamines; nonmotile or motile by means of polar flagella........ **Protaminobacter** p. 65
M p. 200
Not as above...................... **14**

14. Motile by means of polar flagella; straight or curved rods.............. **15**
Motile by means of peritrichous flagella
16

15. Straight rods. **Pseudomonas** p. 61
(*P. melophthora*) *M p. 89*
Curved rods............. **Vibrio** p. 70
(*V. extorquens* and *V. hyphalus*) *M p. 229*

16. Methyl red-positive; Voges-Proskauer-negative........... **Escherichia** p. 79
(*E. aurescens*) *M p. 335*
Bacterium (T and W, 4th ed.)
Methyl red-negative; Voges-Proskauer-positive; pathogenic on rhubarb
Erwinia p
(*E. rhapontica*) *M p.*
Bacterium (T and W, 4th

17. Organisms produce a water-solu
blue, green, or yellow pigment.......
Not as above...........................

18. Agar hydrolyzed; alginic acid dec
posed............ **Alginomonas** p.
(*A. fucicola*) *M p.*

(See also *Pseudomonas gelatica* and *Vi*
fuscus.)

Agar not hydrolyzed
Pseudomonas p.
M p.

19. Plant pathogens................
Not pathogenic or known to be pathoge
to plants..........................

20. Organisms produce hyperplastic
eases such as galls and hairy root
Agrobacterium p.
M p.
Not as above.......................

21. Colonies yellow................
Colonies not yellow................

22. Flagella polar
Xanthomonas p.
M p. 1

(See also *Bacterium tardicrescens, B*
terium albilineans, Pseudomonas
folii, Pseudomonas panicimiliacei, Ps
domonas radiciperda, and *Pseudomon*
levistici.)

Flagella peritrichous.... **Erwinia** p.
M p. 3

Note: The following yellow-pigment
pathogenic organisms are either nonmot
or motility has not been recorded: *B*
terium stewartii, Xanthomonas clerodend
Xanthomonas sesbaniae, and *Coryneb*
terium agropyri.

23. Flagella polar
Pseudomonas p.
M p.

(See also *Xanthomonas panici, X. p*
teamaculans, X. manihotus, X. rubris
balbicans, X. cannae, X. zingiberi, a
X. translucens.)

gella peritrichous.... **Erwinia** p. 93
M p. 349

Bacterium (T and W, 4th ed.)

Curved and irregular cells; may branch oung cultures; capable of using phenolic npounds as the sole source of carbon; , presumably nitrogen, produced in 0.1 cent nitrate broth but no nitrites are duced; no acid from carbohydrates
Mycoplana p. 66
M p. 204

t as above.......................... **25**

Curved rods, very small, about 1.0 μ length, which, during growth, produce sed rings, 2.0 to 3.0 μ in diameter, which er change to two horseshoe-shaped ves fastened together without any evince of divisional lines. These divide into parate rods which reproduce the cycle; capsulated; grow well in 0.5 per cent ptone water.... **Microcyclus** p. 72
M p. 253

ot as above.......................... **26**

. Curved or S-shaped rods, 0.1 to 0.2 by to 8 μ or longer; very poor growth on ptone agar;

or

ort straight rods 0.5 by 1 to 2 μ; good owth on peptone agar; on inorganic osulfate agar, small watery colonies oduced which turn white from the desition of sulfur; grow autotrophically, oxzing thiosulfate to sulfur and sulfate
Thiobacillus p. 60
M p. 83

ot as above.......................... **27**

. Curved cells, 1 by 5 to 10 μ, which rm chains which may twist around each her to form coiled, nonseptate, nonmotile lorless bundles; enclosed in a spherical, lid gelatinous mass from 10 to 17 μ in ameter; found floating on water containg decomposing plant material
Myconostoc p. 73
M p. 260

ot as above.......................... **28**

. Curved organisms which grow on prepitated cellulose-mineral salts agar producing a clearing of the medium around the colonies; also grow in 0.5 per cent peptone water in which a strip of filter paper is half immersed, weakening the filter paper sufficiently for the fibers to separate on slight agitation or reducing it to a pulpy mass; organisms are arranged in palisade fashion similar to the *Corynebacterium* spp.
Cellulomonas p. 114
M p. 601

(See also *Vibrio agarliquefaciens* and *V. fuscus.*)

Not as above.......................... **29**

29. Curved organisms which grow on a filter paper-mineral salts medium, oxidizing the cellulose to oxycellulose...... **30**

Note: There appears to be no justification for recognizing this so-called "oxidation" or for the genera whose existence is based on it; see notes in Methods.

Not as above.......................... **31**

30. Sickle-shaped; cells no longer than 2.0 μ; stain more intensely at the center than at the ends; no growth on a mineral salts agar containing starch; green, cream-colored, or brownish on filter paper
Cellfalcicula p. 72
M p. 252

Curved cells 1.5 to 5.0 μ in length, with rounded ends; grow moderately well on mineral salts agar containing starch; cream-colored, brown, or no pigment on filter paper................ **Cellvibrio** p. 71
M p. 250

31. Curved rods which transform completely into coccoid forms during growth of the colony. Rods elongate and divide. At the point of division the rods grow out at an angle to the original axis and divide again at the angle when the cell has doubled its length. This process continues until a colony is formed. *Ultimately the rods transform completely into cocci*
Arthrobacter p. 114
M p. 605

Note: The emphasis lies on the final transformation into cocci. Some authorities may consider that limited true branching may occur. If this is admitted, the dividing line between *Arthrobacter* and *Nocardia* becomes very slim. The author's observations of *Arthrobacter globiformis* fit the above statement, and it is suggested that these criteria be adopted, with true branching forms which later disintegrate being assigned to *Nocardia*.

Not as above........................ 32

32. Curved rods; motile by means of polar flagella........................ 33

Straight rods........................ 34

33. Oxidize hydrogen sulfide, depositing sulfur inside the cell.. **Thiospira** p. 50

Note: If *Thiospira* should prove to be anaerobic, this insertion would be invalid.

Do not deposit sulfur inside the cell
Vibrio p. 70
M p. 229

34. Acid produced from lactose within 40 days........................ 35

No acid produced from lactose...... 71

35. Agar is digested................ 36

Agar is not digested; organisms parasitic on the cytoplasm of the rhizopod, *Pelomyxa palustris*......*Bacterium* parapelomyxae*
(Not *Bacterium*, T and W, 4th ed.)

Not as above........................ 38

36. Yellow colonies................ 37

Colonies not yellow
Bacterium† nenckii
(*Manual*, 6th ed.)

37. Polar flagella.. **Pseudomonas** p. 61
M p. 89
(Including *P. segnis* and *P. lacunogenes*, placed in the appendix to *Xanthomonas*.)

Motile by means of peritrichous flagella or nonmotile....... **Agarbacterium** p. 88
M p. 322

38. Motile at 37° or at 21° C.......... 39

Nonmotile........................ 59

Motility not recorded (See *Xanthomonas clerodendri* and *X. sesbaniae*.)

39. Acid and gas from glucose.......

Acid only from glucose............

40. Gelatin liquefied...............

Gelatin not liquefied..............

41. Flagella polar; colorless colonies;
mus milk acid, coagulated and slowly
gested; 2,3-butylene glycol produced fr
glucose; cause a fatal septicemia in fish
frogs.............. **Aeromonas** p.
M p.

Flagella polar; colorless colonies; not
above........... **Pseudomonas** p.
M p.

Flagella polar; yellow colonies
possibly **Xanthomonas** p.
(*X. hemmiana*) *M p.*

Flagella peritrichous; colonies colorless.

42. Soft rot produced in sterile plant tissu
Erwinia p.
M p.
Bacterium (T and W, 4th e

Not as above......................

43. Lactose fermented in 2 days.....

Lactose fermentation delayed
Paracolobactrum p.
M p. 3
Paracolon group (T and W, 4th e
(*P. arizonae* and *P. aerogenoides* strai
4611, 1721, and 19111; see also *Erwin*
chrysanthemi and Arizona Group, Rom
1953.)

44. Methyl red-positive; Voges-Proskau
positive or -negative................

Methyl red-negative; Voges-Proskau
positive............ **Aerobacter** p.
M p. 3
Bacterium (T and W, 4th e

45. No gas produced from lactose with
2 days....... **Paracolobactrum** p.
M p. 3
Paracolon group (T and W, 4th e
(*P. intermedium* strains 8011, 13311, a
11411.)

Gas produced from lactose within 2 da
Escherichia p.
M p. 3
Bacterium (T and W, 4th e
(*E. freundii*; See also Arizona Grou
Rome, 1953.)

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* The genus *Bacterium* is not recognized in the *Manual* but in it (pp. 926–930) a few unplaced species in the addendum to Class II still appear under this generic name.

† See Note to H, 35.

Note: Erwinia carnegeana and *Erwinia oseptica* may terminate here.

Flagella polar; litmus milk unchanged; rch hydrolyzed; hydrogen sulfide pro- ced; wide range of sugars fermented; anisms will grow in a nitrogen-free dium fixing atmospheric nitrogen

Azotomonas p. 64
M p. 198

agella polar; acid only in lactose; indole- gative; luminescent on a variety of media pH 8.0...... **Photobacterium** p. 64
M p. 193

Note: The polar-flagellated plant pathons, *Pseudomonas petasitis* (colorless) and *nthomonas conjac* (yellow-pigmented), it is lactose-positive, may terminate here.

ot as above: flagella peritrichous.... **47**

. Alginate fermented with the production of acid and gas; hydrogen sulfide oduced; methyl red-positive; Voges- oskauer-weak; citrate utilized; nitrite oduced from nitrate

Alginobacter p. 93
M p. 348

ot as above........................ **48**

. Methyl red-positive; Voges-Proskauer- gative.......................... **49**

ethyl red-negative; Voges-Proskauer- sitive.......................... **51**

Note: Erwinia nimipressuralis and *E. solvens* should be compared with genera rminating between Sections **49** to **51** in- isive.

. Lactose fermented within 2 days.. **50**

ctose fermentation delayed

Paracolobactrum p. 91
M p. 346

Paracolon group (T and W, 4th ed.)

P. coliforme strains 28221, 5511, 6611, 2611, 31611, 111, 15411, 1811, 33811, and 4361.

P. intermedium strains 14011, 12611, and 1421.

Bethesda and Ballerup Groups, Rome, 1953, for gas-producing strains with slow lactose fermentation.

50. Acid and gas from lactose

Escherichia p. 89
M p. 335

Bacterium (T and W, 4th ed.)
(*Escherichia*, Rome, 1953, for motile strains.)

Acid only from lactose

Paracolobactrum p. 91
M p. 346

Paracolon group (T and W, 4th ed.)
(*P. intermedium* strains 14011 and 12611.)

51. Lactose fermented in 2 days

Aerobacter p. 90
M p. 341

Bacterium (T and W, 4th ed.)

Lactose fermentation delayed

Paracolobactrum p. 91
M p. 346

Paracolon group (T and W, 4th ed.)
(*P. aerogenoides* strains 721, 37711, and 37211.)

52. Organisms cause a glanders-like infection (melioidosis) in rats, guinea pigs, rabbits, and man; colonies on agar small, circular, slightly raised, thick, opaque, and cream-colored with an irregular margin; litmus milk slowly coagulated; blood serum slowly liquefied; optimal temperature, 37° C............ **Pseudomonas** p. 61
(*P. pseudomallei*) *M p. 89*

Organisms which produce granular conjunctivitis in monkeys and apes are believed to be a cause of trachoma in man; optimal temperature, between 28° and 31° C. with motility only at low temperatures; colonies on blood agar small, circular, grayish, and translucent, becoming sticky and mucoid

Noguchia p. 78
M p. 421

Not as above.................... **53**

53. Organisms grow in 0.5 per cent peptone water containing a filter paper strip, weakening the latter sufficiently to cause disintegration on slight agitation or reducing it to a pulp; produce clearings around colonies on precipitated cellulose agar plates; cells commonly arranged in angular fashion like *Corynebacterium* spp.

Cellulomonas p. 114
M p. 601

(See also *Pseudomonas arguta* and *P. tralucida*, and also *P. ephemerocyanea* if the latter produces no pigment.)

Not as above...................... 54

54. Colonies yellow................ 55

Colonies not yellow................ 57

55. Flagella polar. **Xanthomonas** p. 62
M p. 152

(See also *Pseudomonas perlurida*, *P. subcreta*, and *P. cepacia*; see also *P. radiciperda* and *Bacterium tardicrescens* should the latter two be lactose fermenters.)

Flagella peritrichous................. 56

56. Marine organisms; nitrites not produced from nitrates; starch hydrolyzed

Flavobacterium p. 87
M p. 309

Not as above..possibly **Erwinia** p. 93
M p. 349

Bacterium (T and W, 4th ed.)

Note: Erwinia ananas, *E. mangiferae*, *E. vitivora*, and *E. milletiae* terminate here.

57. Organisms found in the cytoplasm of the rhizopod, *Pelomyxa*

Bacterium parapelomyxae*

Note: This entry is valid only if the organism is motile.

Not as above...................... 58

58. Flagella polar. **Pseudomonas** p. 61

Flagella peritrichous

Paracolobactrum p. 91
(Anaerogenic strains) *M p. 346*

Paracolon group (T and W, 4th ed.) Non-gas-producing strains of Ballerup and Bethesda Groups, Rome 1953, which ferment lactose slowly.

Note: From published data the following organisms, if isolated from the soil, may terminate here: *Agrobacterium rhizogenes*, *Erwinia amylovora*, *E. aroidiae*, *Rhizobium leguminosarum*, *R. trifolii*, and *R. phaseoli*. In addition the following may also be listed if they prove to be lactose fermente *Achromobacter delicatulum*, *A. superfici* *Agrobacterium tumefaciens*, *Rhizobium m* *loti*, and *Erwinia salicis*.

59. Acid and gas from glucose.......

Acid only from glucose.............

60. Bioluminescent

Photobacterium p.
M p.

Not as above.......................

61. Methyl red-positive; Voges-Proskau negative.........................

Methyl red-negative; Voges-Proskau positive.........................

62. Lactose fermented in 2 days

Escherichia p.
M p.

Bacterium (T and W, 4th e (Escherichia Group, Rome, 1953, for n motile strains.)

Note: de Bord (Iowa State Coll. J. S *16*, 1942, 471) describes an organism un the name of *Colloides anoxydana* wh terminates here. Isolated from the norm vagina and in cases of vaginitis and c junctivitis, it appears as diplococci on so media on primary isolation but as a mixt of rods and cocci in liquids. It is citra positive and indole-positive.

Lactose fermentation delayed

Paracolobactrum p.
M p.

Paracolon group (T and W, 4th e (*P. coliforme* strains 311, 17611, a 16911.)

63. Encapsulated organisms; pathogen causing infections in man and animals, pr cipally of the respiratory tract

Klebsiella p.
M p.

Bacterium (T and W, 4th ed

Not as above..... **Aerobacter** p.
M p.

Bacterium (T and W, 4th ed (Klebsiella (Klebsiella-Aerogenes) Grou Rome, 1953.)

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* See Note to H, 35.

Small ovoid coccobacilli causing pneu-
nia in sheep and cattle

Pasteurella p. 73
M p. 395

anisms which produce mucoid to car-
ginous colonies adherent to the me-
m; grow on meat infusion agar; colonies
ally 1 mm. in diameter in 24 hours but
y enlarge to 3 to 6 mm. upon further in-
ation; produce a granulated growth on
walls of the tube in liquid media; occur
necrotic lesions as granules resembling
inomycotic granules in cases of actino-
illosis of cattle and joint ill of foals or in
nplicating actinomycotic infections. One
cies may be a commensal in the alimen-
ntary canal of solipeds

Actinobacillus p. 76
M p. 414

t as above...................... **65**

Fastidious organisms; do not grow on
at infusion agar in the absence of X-fac-
on primary isolation under aerobic con-
ions but may be trained to grow without
colonies on blood agar 0.5 mm. in di-
eter but may be much larger on Levin-
l agar; coccobacillary, arranged singly,
short chains, or in clumps

Haemophilus p. 75
M p. 406

Species represented here cause broncho-
neumonia of sheep (*H. ovis*), respiratory
nfection in mice (*H. influenzae-murium*),
and endocarditis (*H. aphrophilus*).

stidious organisms; require blood or
itic fluid; highly pleomorphic; rod-
ped or filamentous; may reach 100 to
) μ in length; homogeneous in young cul-
es but become granulated, beaded, and
ollen in old cultures and tend to frag-
ent into rods and cocci; large spherical
ellings packed with granules common;
olesterol globules appear among the
owth; only rods and short filaments ap-
ar in pathological material; nonbranch-
; nonacid-fast; colonies on ascitic agar
o 2.5 mm. in 3 days, circular, low convex,
lorless; L-phase variants are common; highly virulent for mice, causing polyarthritis. Causes ratbite or Haverhill fever in man......... **Streptobacillus** p. 98
S. (moniliformis) M p. 451

Actinobacillus (*muris*) (T and W, 4th ed.)

Not as above: grow well on media without blood or serum..................... **66**

66. Colonies colorless................ **67**

Colonies yellow.................... **70**

67. Organisms grow in 0.5 per cent peptone containing filter paper and cause the latter to disintegrate; gelatin liquefied; nitrite produced from nitrate; starch hydrolyzed

Cellulomonas p. 114
M p. 601

Not as above...................... **68**

68. Lactose fermented in 2 days...... **69**

Lactose fermentation delayed; enteric pathogens............. **Shigella** p. 96
M p. 384

(Shigella Group, Rome, 1953, slow lactose fermenters.)

69. Encapsulated cells; cause rhinoscleroma.................. **Klebsiella** p. 91
M p. 344

Bacterium (T and W, 4th ed.)

Not as above.... **Achromobacter** p. 87
M p. 300

70. Curved rods which transform completely into coccoid forms during growth of the colony. Rods elongate and divide. At the point of division the rods grow out at an angle to the original axis and divide again at the angle when the cell has doubled its length. This process continues until a colony is formed. *Ultimately the rods transform completely into cocci*

Arthrobacter p. 114
M p. 605

Note: The emphasis lies on the final transformation into cocci. Some authorities may consider that limited true branching may occur. If this is admitted the dividing line between *Arthrobacter* and *Nocardia* becomes very slim. The author's observations of *Arthrobacter globiformis* fit the above statement, and it is suggested that these criteria be adopted, with true branching forms which later disintegrate being assigned to *Nocardia*.

Not as above; starch hydrolyzed

Flavobacterium p. 87
M p. 309

In addition to the above, the following species, two of which are plant pathogens, terminate at this point: *Pseudomonas iridescens*, *Corynebacterium agropyri*, and *Bacterium stewartii*.

71. Nonmotile.................... **72**
Motile.......................... **103**

72. Acid and gas from glucose........ **73**
Acid but no gas from glucose.......... **77**
No acid or gas from glucose.......... **85**

73. Only a small amount of gas from glucose; causes dysentery in man; agglutinated by polyvalent antiserum to *Shigella flexneri*

Shigella p. 96
M p. 384
(Newcastle strain)

Organisms which cause bacillary white diarrhea of chickens; agglutinated with *Salmonella* group D "O" antiserum

Salmonella p. 95
(*S. pullorum*) *M p. 368*

Not as above...................... **74**

74. Bioluminescent on 3 per cent salt agar, especially with a fish base; pleomorphic and branching rods on asparagine-sugar media; 2,3-butylene glycol produced

Photobacterium p. 64
M p. 193

Not bioluminescent................ **75**

75. Gelatin liquefied; nitrites produced from nitrates; methyl red-negative; Voges-Proskauer-negative; starch hydrolyzed; pathogenic to the fish *Salmonidae*

Aeromonas p. 63
M p. 189

Not as above: Voges-Proskauer-positive
76

76. Organisms encapsulated; cause respiratory and other infections in man

Klebsiella p. 91
M p. 344

Bacterium (T and W, 4th ed.)

Not as above.. **Paracolobactrum** p. 91
M p. 346

Paracolon group (T and W, 4th e
(*P. aerogenoides* strains 32011 a
32811.)

77. Obligate halophiles requiring 20 to
per cent salt for growth; pleomorphi
yellow colonies.. **Halobacterium** p.
M p. 2

Not as above....................

78. Organisms will not grow on meat i
fusion agar without the addition of blood
ascitic fluid or X- and V-factors or oth
enrichments......................
Not as above....................

79. Pleomorphic coccobacillary organism
grow well on blood agar producing coloni
up to 4 mm. in diameter on prolonged inc
bation; hydrogen sulfide produced fro
cystine media; slight acid from glucos
fructose, and mannose and possibly fro
other sugars. Pathogenic producing tul
remia or tularemia-like infections in labor
tory animals........ **Pasteurella** p. 7
M p. 39

Brucella (*tularensis*) (T and W, 4th ed
Organisms usually 0.2 to 0.5 μ wide an
0.5 to 2.0 μ long but frequently produc
long filaments; do not grow on nutrien
agar or on MacConkey's lactose bile sa
agar; grow on nutrient agar with the add
tion of X-factor or V-factor or both or wit
the addition of diphosphothiamine c
adenosine triphosphate; colonies on sui
able media rarely more than 1 mm. in d
ameter after 2 days of incubation; nitrite
produced from nitrates; various specie
have been reported as responsible for c
associated with viruses in:

(i) Purulent meningitis and conjuct
vitis in man (*H. influenzae*).
(ii) Subacute endocarditis (*H. hemoly
icus*).
(iii) Acute and subacute conjunctiviti
in man (*H. aegyptius*).
(iv) Soft chancre (*H. ducreyi*).
(v) Vesicular eruptions in the genital
of cattle (*H. citreus*).
(vi) Ulcers of trout (*H. piscium*).
(vii) Commonly present in the respira
tory tract of man (various specie

and preputial secretions of dogs (*H. haemoglobinophilus*).

(viii) Pharyngitis (*H. parahaemolyticus*).

(ix) Nonpathogenic (*H. parainfluenzae*).

(x) Fowl coryza (*H. gallinarum*).

(xi) Swine influenza (*H. suis*).

(xii) Respiratory tract of ferrets (*H. toriorum*) .... **Haemophilus** p. 75 *M p. 406*

Note: This is the main entry for the genus *emophilus*. Owing to variable biochemi- l characteristics, other entries occur in her parts of the Key.

astidious organisms; require blood or as- ic fluid; highly pleomorphic; rod-shaped filamentous; may reach 100 to 150 μ in ngth; homogeneous in young cultures but come granulated, beaded, and swollen in d cultures and tend to fragment into rods d cocci; large spherical swellings packed ith granules common; cholesterol globules pear among the growth; only rods and ort filaments appear in pathological ma- rial; nonbranching; nonacid-fast; colonies ascitic agar 1 to 2.5 mm. in 3 days; circu- r, low convex, colorless. L-phase vari- nts are common. Highly virulent for mice using, polyarthritis; causes ratbite or averhill fever in man

Streptobacillus p. 98 (*S. moniliformis*) *M p. 451*

tinobacillus (*muris*) (T and W, 4th ed.)

Slender rods; 1.0 to 3.0 μ long and often ranged in angular fashion; pleomorphic; oduce smooth, entire, butyrous, trans- cent, grayish yellow colonies 0.5 to 1.0 m. in diameter in 2 days at 37° C. on eat infusion agar; may increase slightly size upon further incubation; light brown n potato; Straus reaction produced in uinea pigs; strict parasites causing glan- ers in man and animals

Actinobacillus p. 76 (*A. mallei*) *M p. 414*

Pfeifferella (T and W, 4th ed.)

mall ovoid coccobacilli arranged singly nd in pairs or in small bundles; sometimes pleomorphic; frequently exhibit bipolar staining; colonies on meat infusion agar 0.1 to 1.0 mm. in diameter in 24 hours at 37° C.; may increase in size up to 4 to 6 mm. in 5 days at 37° C. A slight thin layer or no growth on potato; organisms cause plague in man and rodents or hemorrhagic septicemia in various other animals and in birds............... **Pasteurella** p. 73 *M p. 395*

Organisms cause actinobacillosis in man and other animals and may also be found in actinomycotic lesions; colonies on agar small, circular, bluish gray, translucent, with a smooth surface and an entire edge, up to 1.5 mm. in diameter in 24 hours at 37° C. but increase considerably in size on further incubation; on alkaline potato a slight glistening graying yellow growth is produced........ **Actinobacillus** p. 76 *M p. 414*

Not as above...................... **81**

81. Curved rods which transform completely into coccoid forms during growth of the colony. Rods elongate and divide. At the point of division the rods grow out at an angle to the original axis and divide again at the angle when the cell has doubled its length. This process continues until a colony is formed. *Ultimately the rods transform completely into cocci.* Colonies yellow or colorless....... **Arthrobacter** p. 114 *M p. 605*

Note: The emphasis lies on the final transformation into cocci. Some authorities may consider that limited, true branching may occur. If this is admitted the dividing line between *Arthrobacter* and *Nocardia* becomes very slim. The author's observations of *Arthrobacter globiformis* fit the above statement, and it is suggested that these criteria be adopted, with true branching forms which later disintegrate being assigned to *Nocardia*.

Not as above...................... **82**

82. Colonies yellow

Flavobacterium p. 87 *M p. 309*

Colonies colorless.................. **83**

83. Enteric pathogens of man; agglutinate with *Shigella* antisera.... **Shigella** p. 96
M p. 384

(Shigella Group, Rome, 1953; non-lactose-fermenters.)

Organisms pathogenic to birds; agglutinate with *Salmonella* group D "O" antiserum
Salmonella p. 95
(*S. gallinarum*) *M p. 368*

Organisms which occur as diplococci resembling *Neisseria* on primary isolation on solid media but as a mixture of cocci and rods in liquids, the latter predominating as the culture ages; isolated from normal vagina and from cases of conjunctivitis
Herellea p. 79
(*H. vaginicola*)

Note: This genus does not appear in the *Manual.* See de Bord, Iowa State Coll. J. Sci., *16*, 1942, 471.

Not as above...................... **84**

84. Organisms cause disintegration of filter paper in 0.5 per cent peptone water
Cellulomonas p. 114
M p. 601

Not as above..... **Achromobacter** p. 87
M p. 300

85. Soil organisms; curved rods which transform completely into coccoid forms during growth of the colony. Rods elongate and divide. At the point of division the rods grow out at an angle to the original axis and divide again at the angle when the cell has doubled its length. This process continues until a colony is formed; *ultimately the rods transform completely into cocci*; colonies yellow or colorless
Arthrobacter p. 114
M p. 605

Note: The emphasis lies on the final transformation into cocci. Some authorities may consider that limited, true branching may occur. If this is admitted the dividing line between *Arthrobacter* and *Nocardia* becomes very slim. The author's observations of *Arthrobacter globiformis* fit the above statement, and it is suggested that these criteria be adopted, with true branching forms which later disintegrate being signed to *Nocardia.*

Not as above......................

86. Agar digested
Agarbacterium p.
M p.

Agar not digested................

87. Colonies yellow................

Colonies not yellow................

88. Organisms will grow in a mineral s medium, using CO_2 as a source of carb and oxidizing hydrogen
Hydrogenomonas p.
M p.

Organisms grow in a mineral salts mediu using alkylamines as the sole source carbon and nitrogen
Protaminobacter p.
M p.

Not as above......................

89. Obligate halophiles requiring 20 to per cent salt for growth
Halobacterium p.
M p.

Not as above......................

90. Litmus milk acid or unchanged
Flavobacterium p.
M p.

Litmus milk alkaline..............

91. Nitrites produced from nitrates
Flavobacterium p.
(*F. lutescens* and *F. fucatum*) *M p.*

Nitrites not produced from nitrates
Alcaligenes p.
(*A. marshallii*) *M p.*

92. Loeffler's inspissated serum liquefied

Not as above......................

93. Recorded from septicemia in ducks
Pasteurella p.
M p.

Cause acute ophthalmia (pink eye) cattle; granular conjunctivitis in man, mo keys, and apes and recorded as a possi cause of trachoma in man
Moraxella p.
M p.

Not as above......................

Organisms recorded from an enzootic chronic pneumonia in calves; thin m-negative rods arranged in groups in ues and as coccoid and bacillary forms culture; in the condensation water of od serum, produce mulberry-like flakes to 1 mm. in diameter consisting of cells anged in chains, the latter encased in a stainable material which usually is llen at the tip

Actinobacillus p. 76
(*A. actinoides*) *M p. 414*

t as above....................... **95**

Slender rods; 1.0 to 3.0 μ long, often anged in angular fashion; pleomorphic; duce smooth, entire, butyrous, trans- ent, grayish yellow colonies 0.5 to 1.0 n. in diameter in 2 days at 37° C. on at infusion agar; may increase slightly size upon further incubation; *café au t*-colored on potato; Straus reaction pro- ced in guinea pigs; strict parasite caus- g glanders in man and other animals

Actinobacillus p. 76
(*A. mallei*) *M p. 414*
Pfeifferella (T and W, 4th ed.)

t as above....................... **96**

. Organisms cause brucellosis in man d other animals; often cause abortion in imals; good growth on liver extract agar tryptose phosphate agar; increased CO$_2$ sion necessary for isolation of one ecies; litmus milk alkaline

Brucella p. 75
M p. 404

t as above....................... **97**

. Organisms causing whooping cough in n, principally in children. One species *. pertussis*) will not grow on primary iso- tion on meat extract agar but does not quire X- or V-factor; the other species *. parapertussis*) grows profusely on meat tract agar. Both species grow well on ordet-Gengou medium, producing ooth, raised, entire, pearly, glistening lonies in 48 to 72 hours at 37° C.; nitrites t produced from nitrates; inspissated rum not liquefied. After intraperitoneal injection into mice, death occurs in 2 to 4 days. Autopsy shows extensive hyperemia of the peritoneal wall, infiltration of glands, and the presence of an extremely mucoid exudate in the peritoneal cavity; hemorrhagic necrosis occurs after intradermal inoculation of the rabbit; agglutinated by antisera to *Bordetella pertussis*

Bordetella p. 74
M p. 402
Haemophilus (*pertussis*) (T and W, 4th ed.)

Not as above....................... **98**

98. Organisms usually 0.2 to 0.5 μ wide and 0.5 to 2.0 μ long but frequently produce long filaments; do not grow on nutrient agar without the addition of X-factor or V-factor or both or the addition of diphosphothiamine or adenosine triphosphate; colonies on suitable media rarely more than 1 mm. in diameter after 2 days of incubation; Various species have been reported as responsible for or associated with viruses in:

(i) Purulent meningitis and conjunctivitis in man (*H. influenzae*).
(ii) Subacute endocarditis (*H. haemolyticus*).
(iii) Acute and subacute conjunctivitis in man (*H. aegyptius*).
(iv) Soft chancre (*H. ducreyi*).
(v) Vesicular eruptions in the genitals of cattle (*H. citreus*).
(vi) Ulcers of trout (*H. piscium*).
(vii) The respiratory tract of man (various species) and preputial secretions of dogs (*H. haemoglobinophilus*).
(viii) Pharyngitis (*H. parahaemolyticus*).
(ix) Nonpathogenic (*H. parainfluenzae*).
(x) Fowl coryza (*H. gallinarum*).
(xi) Swine influenza (*H. suis*).
(xii) Respiratory tract of ferrets (*H. putoriorum*).

Of the above, *H. ducreyi* and *H. putoriorum* are the ones most likely to terminate here. The others may do so owing to variation

in biochemical reactions
Haemophilus p. 75
M p. 406

Organisms do not require X- or V-factors. Grow well on meat infusion agar. Occur as diplococci resembling *Neisseria* on agar media and as a mixture of cocci and rods in liquid media, the latter predominating as the culture ages; occur in the normal vagina.................. **Mima** p. 78
(*polymorpha*)

Note: This genus is not listed in the *Manual.* See de Bord, Iowa State Coll. J. Sci., *16*, 1942, 471.

Not as above..................... **99**

99. Organisms grow in a mineral salts medium with an alkylamine as the sole source of carbon and nitrogen
Protaminobacter p. 65
M p. 200

Not as above..................... **100**

100. Organisms found growing in waters among decomposing plant tissue; coccobacillary forms embedded in a gelatinous matrix which usually forms in long fingerlike processes in which the cells are well isolated from each other; produce a zoogloeal mass and cartilaginous colonies in culture.................. **Zoogloea** p. 66
M p. 206

Not as above..................... **101**

101. Litmus milk alkaline........... **102**

Litmus milk acid or unchanged
Achromobacter p. 87
M p. 300

102. Colonies on nutrient agar colorless; up to 1 mm. in diameter in 5 days at 28° C.; grow autotrophically, oxidizing sodium thiosulfate to sodium sulfate and sulfuric acid............. **Thiobacillus** p. 60
M p. 83

Note: This entry applies to *T. novellus* if it produces no acid from carbohydrates.

Not as above...... **Alcaligenes** p. 86
M pp. 297, 1025

'Bacterium' (*alkaligenes*) (T and W, 4th ed.)

103. Acid and gas from glucose...... **104**

Acid but no gas from glucose...... **109**

No acid or gas from glucose....... 1

104. Colonies yellow; flagella polar
Xanthomonas p.
M p. 1

(*X. plantaginis* and *X. conjac,* if lacto
negative, terminate here.)

Colonies not yellow.............. 1

105. Organisms produce a luminesce
growth on agar containing 2.8 to 3.0
cent salt; usually no luminescence on me
with the usual 0.5 per cent salt
Photobacterium p.
M p. 1

Not as above.................... 1

106. Flagella polar; gelatin liquefied; so
species are pathogenic to fish, frogs, a
snakes, whereas others are saprophytic
Aeromonas p.
M p. 1

(See also *Pseudomonas polygoni* and
colurnae (if lactose is not fermented).)

Flagella peritrichous............... 1

107. Gelatin liquefied *or* indole produc
or both; organisms may show a mark
spreading growth on solidified agar with
film of moisture on the surface
Proteus p.
M p. 3

(See also *Paracolobactrum colifor*
strains 4361 and 33811 and Providen
Group, 1953, for aerogenic strains.)

Note: Proteus spp. are probably al
urease-positive. The character is not me
tioned in the descriptions in the *Manu*
but is used in its keys.

Gelatin is not liquefied and indole is n
produced; colonies do not spread on soli
fied agar......................... 1

108. Voges-Proskauer-positive
Paracolobactrum p.
M p. 3

Paracolon group (T and W, 4th e
(*P. aerogenoides* strains 32011, 3771
35611, 37211, 37511, and 32821.)

Voges-Proskauer-negative
Salmonella p.
M p. 3

(Salmonella Group, Rome, 1953.) (S
also *Paracolobactrum intermedium* stra

2611 and 1421 and non-lactose-fer-
nenting strains of the Bethesda-Ballerup
roup, Rome, 1953.)

. Colonies yellow.............. **110**

lonies not yellow................ **113**

. Bioluminescent on media containing
er cent salt...**Photobacterium** p. 64
M p. 193

t as above...................... **111**

. Chitin is hydrolyzed
Beneckea p. 89
M p. 328

t as above...................... **112**

2. Flagella polar
Xanthomonas p. 62
M p. 152

(See *Pseudomonas xantha* and *P. pictorum* and also *P. trifolii*, *P. radiciperda*, and *Bacterium tardicrescens* if they produce no acid from lactose.)

agella peritrichous
Flavobacterium p. 87
M p. 309

hromobacterium (T and W, 4th ed.)

Note: The following plant pathogens also
rminate at this point: *Erwinia cassavae*,
citrimaculans and *Agrobacterium gypso-*
ilae.

3. Animal parasites; colonies mucoid;
lated from the eye of the Rhesus mon-
y..................**Noguchia** p. 78
M p. 421

nall translucent colonies; mucoid, small
nder rods in smooth colonies; ovoid rods
th bipolar staining in rough colonies;
otile at 37° C.; organisms cause melio-
osis in man and other animals
Pseudomonas p. 61
(*P. pseudomallei*) *M p. 89*

feifferella (*whitmori*) (T and W, 4th ed.)

Note: This insertion covers strains which
ve lost their ability to produce acid from
ctose.

nall umbonate colonies, translucent with
dull, finely granular, "beaten copper"
rface, entire, butyrous; organisms cause
pseudotuberculosis in rodents
Pasteurella p. 73
M p. 395

Organisms found in the cytoplasm of the rhizopod, *Pelomyxa palustris*
Myxococcus
(*M. pelomyxae*) *M p. 930*

Not as above..................... **114**

114. Organisms cause typhoid fever in man; agglutinated by *Salmonella* group D "0" antiserum when smooth but not in the "Vi" phase; flagella peritrichous
Salmonella p. 95
(*S. typhosa*) *M p. 368*

Not as above..................... **115**

115. Agar is digested
Agarbacterium p. 88
M p. 322

Agar is not digested............... **116**

116. Chitin is hydrolyzed
Beneckea p. 89
M p. 328

Chitin not hydrolyzed............. **117**

117. Bioluminescent on media containing 3 per cent salt; may not be luminescent on 0.5 per cent salt
Photobacterium p. 64
M p. 193

Not as above..................... **118**

118. Flagella polar
Pseudomonas p. 61
M p. 89

(See also *Xanthomonas manihotis*, *X. proteamaculans*, and *Rhizobium meliloti* (if lactose-negative).)

Flagella peritrichous............... **119**

119. Cause hyperplastic diseases of plants
Agrobacterium p. 85
M p. 288

(*A rubi*, *A. tumefaciens* (if lactose-negative) and *A. pseudotsugae*.)

Cause wilts or necrotic diseases of plants
Erwinia p. 93
M p. 349
Bacterium (T and W, 4th ed.)

(*E. amylovora*, *E. tracheiphila*, and *E. salicis* (if lactose-negative).)

Not as above.....**Achromobacter** p. 87
M p. 300

Note: Some anaerogenic paracolons terminate here (strains 33111, 29911, and 8911). See also anaerogenic strains of the Providence Group, Rome, 1953.)

120. Curved rods which transform completely into coccoid forms during growth of the colony. Rods elongate and divide. At the point of division the rods grow out at an angle to the original axis and divide again at the angle when the cell has doubled its length. This process continues until a colony is formed. *Ultimately the rods transform completely into cocci*
Arthrobacter p. 114
M p. 605

Note: The emphasis lies on the final transformation into cocci. Some authorities may consider that limited true branching may occur. If this is admitted the dividing line between *Arthrobacter* and *Nocardia* becomes very slim. The author's observations of *Arthrobacter globiformis* fit the above statement, and it is suggested that these criteria be adopted, with true branching forms which later disintegrate being assigned to *Nocardia.*

Organisms which will grow in a mineral salts medium, using phenol as the sole source of carbon; reduce nitrates with the liberation of gas, presumably nitrogen
Mycoplana p. 66
M p. 204

Not as above **121**

121. Colonies yellow **122**

Colonies not yellow **126**

122. Agar hydrolyzed
Agarbacterium p. 88
M p. 322

Agar not hydrolyzed **123**

123. Organisms grow in a mineral salts base, using CO_2 as the sole source of carbon and oxidizing hydrogen
Hydrogenomonas p. 58
M p. 75

Not as above **124**

124. Flagella polar
Xanthomonas p. 62
M p. 152

(See also *Pseudomonas lasia, P. cerev*
siae, P. caudata, P. levistici, P. ochrace
and *P. panicimiliacei.*)

Flagella peritrichous **12**

125. Litmus milk strongly alkaline
Alcaligenes p. 8
M p. 29

(See also *Flavobacterium harrisonii.*)

Litmus milk acid or unchanged
Flavobacterium p. 8
M p. 30

Chromobacterium (T and W, 4th ed

126. Pleomorphic rods; do not grow o
meat infusion agar; grow well in semisoli
media containing horse, rabbit, or huma
blood or other complex substances; rod
and coccoid forms predominate in culture
flagella polar; pathogenic to man; multipl
on erythrocytes and in fixed tissue cells
transmitted through the sandfly, *Phlebot*
mus verrucarum ...... **Bartonella** p. 14
M p. 96

Colonies mucoid, small, circular and trans
lucent; organisms cause conjunctival fol
liculosis in rabbits ..... **Noguchia** p. 7
M p. 42

Small, smooth, raised, entire, pearly colo
nies on meat infusion agar; medium dis
colored; litmus milk alkaline in 1 to 2 days
cause bronchopneumonia in rodents an
sometimes associated with canine distem
per .... **Bordetella** p. 7
M p. 40

Haemophilus (T and W, 4th ed

Not as above **12**

127. Agar digested **12**

Agar not digested **12**

128. Alginates are metabolized; flagell
polar .... **Alginomonas** p. 6
M p. 20

(See also *Pseudomonas gelatica.*)

Alginate metabolism not recorded; flagell
peritrichous ..... **Agarbacterium** p. 8
M p. 32

129. Obligate halophiles, requiring 20
30 per cent salt for growth
Halobacterium p. 6
M p. 20

Not as above **1**

). Alginates metabolized; flagella polar **Alginomonas** p. 65
M p. 202

t as above.......................... **131**

1. Organisms found growing in water ong decomposing plant tissue; produce haracteristic lobed gelatinous zoogloeum which the cells are well isolated from ch other; produce cartilaginous colonies agar................**Zoogloea** p. 66
M p. 206

lls actively motile, by means of polar gella; deposit sulfur internally when owing in the presence of hydrogen sulfide **Thiospira** p. 59
M p. 82

Note: It is doubtful whether this genus ould appear under aerobic organisms.

ot as above........................ **132**

2. Flagella polar.................. **133**

agella peritrichous................ **135**

3. Organisms will grow in a mineral salts edium, using CO_2 as the sole source of rbon and will oxidize hydrogen **Hydrogenomonas** p. 58
M p. 75

ods 0.5 by 1 to 2 μ; gelatin liquefied; itrites and gas produced from nitrates; arch hydrolyzed; lipolytic; will grow autoophically, oxidizing thiosulfate to sulfate d tetrathionate with an increase in pH. lfur is not precipitated **Thiobacillus** p. 60
M p. 83

ot as above....................... **134**

4. Organisms cause disintegration of ter paper when growing in 0.5 per cent eptone water.... **Cellulomonas** p. 114
M p. 601

(See also *Pseudomonas mira.*)

ot as above..... **Pseudomonas** p. 61
M p. 89

(See also *Agrobacterium stellulatum*, *A. radiobacter*, *Xanthomonas panici*, *X. rubrisubalbicans*, *X. cannae*, *X. zingiberi*, and *Rhizobium japonicum*.)

35. Litmus milk alkaline **Alcaligenes** p. 86
M p. 297

'Bacterium' (*alkaligenes*) (T and W, 4th ed.)

(See also *Agrobacterium radiobacter* and *Rhizobium* spp.)

Litmus milk acid or unchanged **Achromobacter** p. 87
M p. 300

SECTION I

1. Endospores produced.............. **2**

No endospores produced.............. **3**

2. Long thin rods oxidizing primary alcohols to acids and secondary alcohols to ketones while reducing CO_2 quantitatively to methane; glucose is not metabolized **Methanobacterium** p. 71
(*M. omelianskii*) *M p. 250*

Not as above...... **Clostridium** p. 116
M p. 634

3. Motile.......................... **4**

Nonmotile........................ **5**

4. Curved rods with a bunch of flagella inserted at or near the center of the concave side of the rod; recorded from the alimentary canal of ruminants and guinea pigs and from the buccal cavity of man **Selenomonas** p. 73
M p. 258

Curved rods which reduce sulfates, sulfites, sulfur, thiosulfate, and hyposulfites to hydrogen sulfide, using a wide range of organic substances and possibly hydrogen as hydrogen donors; motile by means of polar flagella...... **Desulfovibrio** p. 70
M p. 248

Note: Desulfovibrio desulfuricans has been recorded as a spore former, but this claim has now been withdrawn.

Curved rods depositing sulfur internally when growing in the presence of hydrogen sulfide; motile by means of polar flagella **Thiospira** p. 59
M p. 82

Curved rods other than above; motile by means of polar flagella..... **Vibrio** p. 70
M p. 229

Note: V. sputorum and *V. niger* are the only two species recorded here. *V. coli* requires 15 per cent CO_2 but is not recorded as anaerobic.

Not as above........................ 5

5. Organisms which ferment glucose with the production of large quantities of ethyl alcohol plus other byproducts including CO_2.............. **Zymomonas** p. 65
M p. 199

Rods straight or slightly curved; occur in liquids in long chains, frequently in parallel bundles; ferment acetate and *n*-butyrate with the production of methane and carbon dioxide; ethyl alcohol and *n*-butyl alcohol not fermented
Methanobacterium p. 71
(*M. soehngenii*) *M p. 250*

Not as above........................ 6

Note: The only recorded motile Gram-negative rods are *Fusobacterium praeacutum*, *Bacteroides serpens*, and *Bacteroides variegatus*. They are treated with other members of their respective genera.

6. Minute rod-shaped cells growing only in media containing sterile fresh tissue or ascitic fluid; found in the upper respiratory tract of man........... **Dialister** p. 97
M p. 440

'Bacterium' *pneumosintes* (T and W, 4th ed.)

Not as above........................ 7

7. Acid and gas produced from glucose *or* acid from glucose and gas produced in peptone water........................ 8

Acid but no gas produced from glucose.. 12

No acid or gas produced from glucose.. 20

Note: No sugar reactions are given for the following species: *Sphaerophorus gonidiaformans, S. mortiferus, S. floccosus, S. influenzaeformis,* and *S. pyogenes*; and *Bacteroides distillationis, B. viscosus,* and *B. glutinosus*

8. Lactose fermented................. 9

Lactose not fermented............... 10

9. Gas produced in peptone; very pleomorphic............ **Sphaerophorus** p. 97
M p. 441

(*S. freundii, S. ridiculosis, S. necroticus,* and *S. inequalis*; see also *Bacteroides serpens.*)

No gas produced in peptone; pleomorp forms not uncommon
Bacteroides p.
M p.

(See also *Sphaerophorus gulosus.*)

10. Halophilic, requiring more than 4 cent salt for growth; gas produced fr peptone............ **Bacteroides** p.
(*B. halosmophilus*) *M p.*

Not halophilic.......................

11. Very pleomorphic; gas produced fr peptone......... **Sphaerophorus** p.
M p.

(*S. necrogenes* and *S. necrophorus* a possibly *S. varius* and *S. bullosus.*)

Note: S. necrophorus is placed in the ger *Fusiformis* in Topley and Wilson, 4th e tion.

Bifurcated cells; gas production from p tone doubtful....... **Bacteroides** p.
(*B. furcosus*) *M p.*

12. Acid produced from lactose.......

No acid produced from lactose........

13. Motile.......... **Bacteroides** p.
(*B. variegatus*) *M p.*

Nonmotile............................

14. Nonpleomorphic, *fusiform* cells gro ing poorly on plain agar (?)
Fusobacterium p.
M p.

Not as above.........................

15. Gelatin liquefied; very pleomorphic ordinary or blood agar
Sphaerophorus p.
(*S. glycolyticus* *M p.*
and................ **Bacteroides** p.
(*B. uncatus*) *M p.*

Gelatin liquefied; not pleomorphic
Bacteroides p.
M p.

(*Bacteroides exiguus, B. uniformis,* *tumidus,* and some strains of *B.* *tasonis*)

Gelatin not liquefied **Bacteroides** p.
M p.

16. Motile; fusiform rods; pleomorphic
Fusobacterium p.
(*F. praeacutum*) *M p.*

Nonmotile............................

Very small rods capable of passing
ugh a Chamberland L_2 filter; acid pro-
d from glucose, sucrose, and mannitol;
rded from the respiratory tract of man

Dialister p. 97
M p. 440

as above........................ **18**

Organisms dependent on serum or
tic fluid for growth; pleomorphic

Sphaerophorus p. 97
floccosus and *S. abscedens*) *M p. 441*

as above........................ **19**

Slender, pointed rods

Fusobacterium p. 97
polymorphum and *F. vescum*) *M p. 436*

ls with rounded ends

Bacteroides p. 96
M p. 424

B. cylindroides, B. capillosus, B. fragilis,
. perfoetens, B. uncatus, and *B. suc-*
inogenes.)

Acid from fructose

Sphaerophorus p. 97
(*S. siccus*) *M p. 441*

t as above........................ **21**

Organisms require serum or ascitic
d for growth; pleomorphic

Sphaerophorus p. 97
(*S. caviae*) *M p. 441*

as above; not pleomorphic

Bacteroides p. 96
M p. 424

B. putidus, B. putredinus, and *B. coagu-*
ns.)

SECTION J

Cells occur singly; motile by means of
ar flagella; may be encapsulated and
y form zoogloeae but if so, do not oxidize
drogen sulfide and do not deposit sulfur
ernally........................ **2**

lls occur singly, in pairs or in short
ins; nonmotile; individual cells encap-
ated and usually contain two pseudo-
uoles per cell which make them buoyant
l which may given them a polygonal
earance; sulfur is deposited internally

Rhodothece p. 51
M p. 50

Cells occur in the natural habitat arranged radially to form rosettes and reassemble in this form after mechanical dispersion. In peptone-yeast extract and other media they occur singly or in small groups. Rosettes may be 15 to 17 μ in diameter; individual cells 1 by 4 to 6 μ; motile; do not deposit sulfur internally....... **Vannielia** p. 55

Note: This genus is not listed in the *Manual.* See Pringsheim, J. Gen. Microbiol., *13*, 1955, 285.

Cells occur in well defined aggregates. When growing in the presence of hydrogen sulfide, sulfur is deposited internally... **4**

2. Hydrogen sulfide is not oxidized. Sulfur is not deposited internally; do not aggregate in rosettes

Rhodopseudomonas p. 54
M p. 53

Hydrogen sulfide is not oxidized; sulfur is not deposited internally; cells occur in their natural habitat arranged radially to form rosettes and reassemble in this form after mechanical dispersion. In peptone-yeast extract and other media they occur singly or in small groups. Individual cells 1 by 4 to 6 μ; motile....... **Vannielia** p. 55

(For reference see J, **1**.)

Hydrogen sulfide is oxidized, and globular sulfur is deposited internally.......... **3**

3. Cells of uneven width and length; often swollen, spindle-shaped and filamentous

Rhabdomonas p. 52
M p. 48

Cells of uniform width; spherical to cylindrical, the latter sometimes slightly curved

Chromatium p. 52
M p. 50

Note: Single cells of *Thiothece, Thiocystis,* and *Lamprocystis* resemble *Chromatium* very closely. Also cells of *Thiospirillum violaceum* are indistinguishable from curved cylindrical forms of *Chromatium.*

4. Cells occur in cubical packets

Thiosarcina p. 48
M p. 39

Cells in young colonies appear as flat sheets in which cells are arranged in parallel rows embedded in a capsular material. In an unfavorable position or environment, irregular clumping of cells may occur

Thiopedia p. 48
M p. 40

Other than above.................... 5

5. Individual cells or cell masses embedded in conspicuous capsules or zoogloea.... 6

Individual cells or cell masses apparently devoid of capsular material, although cells within the mass may be clearly separated in space........................ 7

6. Cells spherical, occurring in large numbers well separated in a conspicuous common slime capsule. In dry conditions the capsular material forms a double contoured membrane around the cells. On moistening, the mass slowly swells and bursts. As the liberated cells divide they form a flat, spreading colony in which the cells are no more than three layers deep but are separated from each other by capsular material

Thiocapsa p. 49
M p. 41

Cells spherical and encapsulated. In the early stages of growth of the colony, cells form in tetrads* resembling those of *Thiocystis*. Further division of the cells gives rise to a compact opaque mass of cocci embedded in a large slime capsule. This is followed by formation of arched fragments similar in appearance to sections of a hollow sphere, the fragments being arranged as if on the surface of a sphere within the capsule. Continued growth of the arched fragments results in their edges touching but not coalescing. Infolding of the arched pieces takes place, finally almost filling the internal cavity and producing a network resembling a sponge. The enveloping capsule eventually ruptures, liberating fragments of the zoogloeal network which reorganize into small groups interlinked by single cocci. Under unfavorable conditions the cocci are vacuolated, and sulfur is confined to the peripheral cytoplasm. Free
cells are motile (*cf. Chromatium*). The tot
transformation occurs in 40 days

Lamprocystis p.
M p.

Cells spherical to cylindrical. Develop
small clusters, the compact clusters of 4
20 cells being widely separated in an almo
spherical and seemingly cartilaginous ca
sule, the outer layer of which is neith
sharply differentiated nor obviously de
quescent. As each cluster proliferates,
eventually separates into a number
smaller clusters *within the zoogloea*. Sing
cells are rare. At some stage individu
clusters leave the mass as a result of eith
swelling and dispersion of the whole ma
or a softening of the mass at one or mo
points. After separation, the cluster b
comes motile. The process of separation
clusters requires 3 weeks. Generation tim
is approximately 2 days

Thiocystis p. 5
M p. 4

Cells spherical to cylindrical; heavily en
capsulated with the capsules remaining a
tached to form zoogloea. The individu
capsules are very thick. After division th
cells become separated in space by the de
veloping capsules and are eventually a
ranged at an obtuse angle to each othe
Swarming is preceded by a softening of th
capsule and an irregular rearrangement
the cells. Individual cells separate by
slow rotatory action and once free are a
tively motile (*cf. Chromatium*). Cells ar
pale gray. Sulfur granules are small an
confined to the peripheral layer of cyt
plasm.............. **Thiothece** p. 4
M p. 4

7. Cells spindle-shaped; 1.5 to 1.7 by 2
to 5.0 μ. Cell families may consist of a lon
irregular body made up of cells 2 to 3 laye
thick arranged in parallel fashion. Whe
separated from other cell masses the cel
rearrange to form an open pyramidal ne
work in which the cells meet only at the
tips. Colonies may be several hundred m
crons across and resemble *Hydrodictyo*
the green algae. Small cell groups d

---

*Winogradsky considered that cells which form tetrads divide in *three* directions, the result being a 4-pointed group rather than a flat group of 4.

ed from the mass are motile; compact
sses form under unfavorable conditions.
ividual cells contain an elongated
uole and are pale in color; sulfur is
fined to the peripheral cytoplasm
Thiodictyon p. 49
M p. 41

ls compressed into a compact mass;
ored rose red; surrounded by a capsule
nposed of an inner, poorly refracting
er and an outer, strongly refracting
er. Placed in a favorable environment,
cyst cracks, and the cell mass slowly
eps out (1 to 2 days). The empty cyst
nains unchanged for some time. The
up of cells does not disperse but grows
an irregularly contoured mass. Whole
nilies are motile. Most cells are spherical.
vision occurs in only one direction. Cells
y be compressed or may be freely sepa-
ed in the nonencysted state. There are
ely more than two to four within a
oup in the colony. They are continually
anging position and proximity in the
ony. Internal colonial movement can
cur without much lateral movement. Co-
linated lateral movement is amoeboid.
ngle cells occasionally become isolated
m the mass and then are drawn back.
sses ultimately may separate into
aller groups. No capsular material can
discerned in the families. Families can
penetrated by small, motile bacteria
h ease. Winogradsky considered that
connection was via plasma threads, but
was unable to demonstrate them. He
nsidered the dense compact masses to
ur in the presence of O_2 and the open
ucture in the presence of hydrogen sulfide
Amoebobacter p. 50
M p. 44

apeless, thick aggregates of small, spher-
al, vividly colored cells; nonmotile; do not
m hollow spherical structures. The ex-
mely smooth surface of the colony sug-
sts a limiting capsular material, but no
vious capsule is visible. Eventually
owth, in the form of threads and flaps,
pears on the surface, and the cells become separated. **Thiopolycoccus** p. 51
M p. 45

SECTION K

The keys of this Section have been reproduced and modified in this form with the permission of the publishers and authors, Dr. C. B. Philip, Dr. C. W. Rake, and Dr. D. Weinman.

1. Parasites, intracellular or intimately associated with tissue cells other than erythrocytes or with certain organs in arthropods; rarely extracellular in arthropods.............................. 2

Parasites, intracellular or facultatively extracellular; found characteristically in or on the erythrocytes of vertebrates, exceptionally in fixed-tissue cells........... 13

2. Frequently cause diseases of vertebrates. Transmitted by arthropod vectors 3

Intracellular parasites found in tissues of vertebrates. Not known to be transmitted by arthropod vectors................ 9

3. Adapted to existence in arthropods; vertebrate hosts include man; cells rod-shaped, ellipsoidal, coccoid, and diplococcoid; rarely filamentous........................ 4

Only a few species adapted to invertebrate existence; pathogenic for certain mammals but not for man; cells spherical, occasionally pleomorphic................ 5

Adapted to existence in arthropods as symbiotes but not in vertebrates as highly pathogenic parasites; cells pleomorphic, coccoid to short or long and curved rods, or even filamentous.................... 7

4. Nonfilterable; produce typhus-like rash and usually Proteus X (Weil-Felix) agglutinins in man......**Rickettsia** p. 138
M p. 935

Filterable; produce neither rash nor Weil-Felix agglutinins in man. . **Coxiella** p. 138
M p. 947

5. Transmitted by ticks............. 6

Transmitted by parasitic trematodes;

pathogenic principally for canines
Neorickettsia p. 139
M p. 951

6. Transmitted transovarially; parasites of circulating monocytes of vertebrate hosts
Ehrlichia p. 139
M p. 949

Not transmitted transovarially; parasites of endothelial cells of vertebrate hosts
Cowdria p. 139
M p. 950

7. No known filterability; no reported association with intracellular crystalline inclusions **8**

Filterable; cause blue disease of beetle larvae; associated with intracellular, crystalline inclusions; reportedly invade cell nuclei . . . . **Rickettsiella** p. 139
M p. 957

8. Symbiotic to highly pathogenic; no mycetomes produced in hosts
Wolbachia p. 139
M p. 953

Symbiotic to the point that special mycetomes are developed for harboring the organisms, which are not pathogenic, in the host . . . . **Symbiotes** p. 139
M p. 956

9. Non cultivable in chicken embryonic tissues **10**

Cultivable in chicken embryonic tissues
Miyagawanella p. 140
M p. 961

10. Organisms coccoid; do not exhibit pleomorphism . . . . **Chlamydia** p. 140
M p. 958

Organisms usually coccoid or ellipsoidal exhibit marked pleomorphism **11**

11. Pleomorphic forms small (200 mμ to 2 μ); pathogenic **12**

Pleomorphic forms large (2μ). Apparently nonpathogenic; may be saprophytic
Colettsia p. 140
M p. 961

12. Occur intracytoplasmically as prominent colonies . . . . **Colesiota** p. 140
M p. 959

Occur intracytoplasmically as scattered
growth . . . . **Ricolesia** p.
M p.

13. Small, rod-shaped, bacteria-like c
At least one species, when cultured, r
show a single, polar flagellum. Arthro
transmission established for some speci

Very small, virus-like particles occurrin
the erythrocytes of vertebrates. Tr
mitted by arthropods. . **Anaplasma** p.
M p.

14. Multiply on erythrocytes and wit
fixed-tissue cells. Usually possess a sin
polar flagellum when cultivated in or
nonliving media. Provoke a progres
anemia or a cutaneous eruption, usu
both in succession, not both coincidenta
Found in man and *Phlebotomus spp.*
Bartonella p.
M p.

Not known to multiply in fixed-tissue ce
parasitize erythrocytes and may multi
there. Flagella not demonstrated. Occu
mammals and possibly in other vertebra
but not known from man . . . .

15. Usually parasitize less than 5 per c
of the total erythrocytes, rarely mo
Relatively monomorphic in erythrocy
Nonpathogenic or only slightly so. Affec
little, if at all, by splenectomy. Cultiva
on nonliving media. Occur within the
blood cells; epierythrocytic forms are pr
lematical . . . . **Grahamella** p.
M p.

Parasitized cells may constitute more t
90 per cent of the total erythrocytes at
peak of infection. Polymorphism is mar
when in or on red blood cells. May or n
not be pathogenic. Marked increase
numbers after splenectomy. Cultivation
nonliving media not confirmed. Occur
the red blood cells; situation within
cells possible but not proved . . . .

16. Extremely polymorphic; however, r
of varying sizes almost invariably oc
often in chains. Habitat predominan
epierythrocytic. Usually pathogenic, p
voking a progressive, sometimes fa

nia...... **Haemobartonella** p. 141
M p. 972

damental morphological type is ring- isc-shaped. Rods are one disc- or ring- ıeter in length; composite rods are e of these units. Occur in great numbers he blood plasma as well as on the hrocytes. Usually nonpathogenic
Eperythrozoon p. 141
M p. 977

SECTION L

he keys in this section have been repro- ed and modified in this form with the nission of the publishers and the author, R. Y. Stanier.

Neither fruiting bodies nor resting cells luced........... **Cytophaga** p. 132
M p. 858

ting cells produced................ **2**

Resting cells cylindrical, not spherical llipsoidal. Fruiting bodies produced.. **3**

ting cells (microcysts) spherical or ellip- lal, surrounded by a distinct wall. iting bodies formed except in the genus *rocytophaga*...................... **9**

Resting cells not contained in cysts. iting bodies consist of mesenteric masses nger-like aggregations of resting cells
4

ting cells contained in cysts of definite pe borne on the fruiting bodies...... **5**

Fruiting body depressed, usually irregu- y delimited, the interior usually con- ng of swollen or intestine-like twisted or rtwined masses, the windings of which y be constricted or may jut out (project) free ends....... **Archangium** p. 132
M p. 863

iting body consists of single (separate) columnar or finger-like structures arising from the substrate.. **Stelangium** p. 133
M p. 866

5. Cysts angular. Vegetative cells always thick and short, with blunt, rounded ends
Sorangium p. 133
M p. 866

Cysts rounded. Vegetative cells usually long and thin, with tapering ends...... **6**

6. Cysts embedded in slime; sessile, occurring singly or as loose aggregates
Polyangium p. 133
M p. 870

Cysts never embedded in slime; either borne on stalks or arranged in tight clusters joined together at the base............ **7**

7. Many cysts united at base to form a large disc or rosette; either sessile or stalked
Synangium p. 134
M p. 877

Cysts not united at base; borne singly or in large numbers on stalks............... **8**

8. Cysts borne singly on a stalk
Podangium p. 134
M p. 877

Numerous cysts on a stalk
Chondromyces p. 134
M p. 879

9. Definite fruiting bodies formed..... **10**

No definite fruiting bodies
Sporocytophaga p. 136
M p. 890

10. Microcysts not enclosed in larger cysts
11

Microcysts enclosed in large cysts
Angiococcus p. 135
M p. 889

11. Fruiting bodies deliquescent
Myxococcus p. 135
M p. 883

Fruiting bodies firm, not deliquescent
Chondrococcus p. 135
M p. 886

DIGEST OF GENERA

PHOTOSYNTHETIC BACTERIA

RED AND PURPLE SULFUR BACTERIA

GENERAL NOTES: The following genera are all colored *red* or *purple* or, if they contain large quantities of sulfur, may be peach-colored. The color is due to carotenoids pigments which predominate over the green *bacteriochlorophyll*. In their natural habitat these genera have been found in waters containing H_2S and so far as is known from the isolated species, namely of *Chromatium*, they develop only under anaerobic conditions exposed to light in the presence of H_2S or other suitable hydrogen donor. The H_2S (HS^-) is oxidized to S^0 which is deposited internally.

Those species which are motile, with the exception of *Amoebobacter*, have polar flagella.

When examined spectroscopically they show a band of absorption at 590 mμ, which corresponds to the *bacteriochlorophyll*, and others at the shorter wave lengths due to carotenoids. Upon extraction with alcohol the band at 590 mμ disappears and is replaced by another at approximately 774 mμ. The shift is possibly due to the dissociation of the chromoprotein.

Sulfur can be extracted with acetone or pyridine.

Most of the genera have been described from slide cultures of crude material by Winogradsky and generic differences lie mainly in the state of aggregation of the cells. More recent observations by van Niel (Bacteriol. Revs., *8*, 1944, 1) suggest that at least some of the genera may represent only different environmental reactions of a single genus *Chromatium*. Others, because of their particular mode of division, are quite distinct. Single cells of *Thiothece*, *Thiocystis*, and *Lamprocystis* resemble *Ch* *matium* very closely. Also cells of *Th* *spirillum violaceum* are indistinguisha from the curved forms of *Chromatium*.

Van Niel found that *Thiocystis*-like pu ple bacteria varied in size from 7 μ at p 7.2 to 1.5 μ at pH 9.0 in the presence of 0. per cent H_2S. They also varied in si with increase within limits, in the sulfi concentration. At high pH, forms rese bling *Thiothece*, *Thiocystis*, and *Thiocap* were unmistakable. At low pH, forms sim lar to *Thiosarcina* and *Thiopedia* were pr duced.

This group of genera is morphologica heterogeneous and does not, in the autho opinion, form a true taxonomic group. Th are placed in the family *Thiorhodaceae* the *Manual*.

In the following genera all those a tributed to Winogradsky were describ in Zur Morphologie und Physiologie d Bacterien, I. Schwefelbacterien., Leipz 1888.

Thiosarcina (Thi.o.sar.ci′na) Wi gradsky, 1888. *M p. 39.*

DIFFERENTIATING CHARACTERS: Unicel lar, spherical organisms, 2 to 3 μ in dian ter, occurring in cubical packets. They co tain bacteriochlorophyll and caroten pigments with predominance of the lat so that the cell masses are colored vari shades of red or purple. When growing the presence of H_2S they deposit sulfur ternally.

Type species: *Thiosarcina rosea* (Schr ter) Winogradsky.

NOTES: Only one species is described. (S also general notes, p. 48.)

Thiopedia (Thi.o.ped′ia) Winogr sky, 1888. *M p. 40.*

FFERENTIATING CHARACTERS: Unicellu-
spherical organisms, 1 to 2 μ in diame-
Under optimal conditions the cells in
ng colonies are arranged in chains which
r in parallel bundles in flat sheets and
embedded in a capsular material. These
ns resemble *Merismopedia* of the blue-
n algae. Under unfavorable conditions
typical arrangement may be lost. The
nisms contain bacteriochlorophyll and
tenoid pigments, and the latter pre-
inate to color the cell masses various
les of red or purple. When growing in
presence of H_2S they deposit sulfur in-
nally.

ype species: *Thiopedia rosea* Winograd-
.

OTES: Only one species has been de-
bed. (See also general notes, p. 48.)

hiocapsa (Thi.o.cap′ sa) Winograd-
, 1888. *M p. 41.*

IFFERENTIATING CHARACTERS: Unicellu-
spherical organisms, 2.5 to 3.0 μ in di-
eter, occurring in large numbers, well
arated in a conspicuous common slime
sule. In dry conditions the capsular ma-
al forms a double contoured membrane
und the cells. Upon moistening the mass
vly swells and bursts. As the liberated
s divide they form a flat spreading col-
in which the cells are no more than
e layers deep but are separated from
n other by a capsular material. Indi-
ual cells or cell masses are embedded
onspicuous capsules or zoogloea. Con-
bacteriochlorophyll and carotenoid
nents and the latter predominate to
r the cell masses various shades of red
urple. When growing in the presence of
S they deposit sulfur internally.

ype species: *Thiocapsa roseopersicina*
nogradsky.

OTES: Only one species has been de-
ibed. (See also general notes, p. 48.)

hiodictyon (Thi.o.dic′ ty.on) Wino-
dsky, 1888. *M p. 41.*

IFFERENTIATING CHARACTERS: Unicellu-
spindle-shaped organisms, 1.5 to 1.7 μ
2.5 to 5.0 μ. Cell families may consist of a long irregular body made up of cells 2 to 3 layers thick, arranged in parallel fashion. When separated from other cell masses, the cells rearrange to form an open pyramidal net in which the cells meet only at their tips. Colonies may be several hundred microns across and resemble *Hydrodictyon* of the green algae. Small cell groups detached from the mass are motile. Compact masses form under favorable conditions. Individual cells contain an enlongated vacuole, are pale in color, and sulfur is confined to the peripheral cytoplasm. Individual cells or cell masses are apparently devoid of capsular material, although cells within the mass may be clearly separated in space. The organisms contain bacteriochlorophyll and carotenoid pigments; the latter predominate to color the cell masses various shades of red or purple. When growing in the presence of H_2S they deposit sulfur internally.

Type species: *Thiodictyon elegans* Winogradsky.

NOTES: Only one species is described. (See also general notes, p. 48.)

Thiothece (Thi.o.the′ ce) Winogradsky, 1888. *M p. 42.*

DIFFERENTIATING CHARACTERS: Unicellular, spherical to cylindrical organisms, 4 to 6 μ by 4 to 7 μ, heavily capsulated with the capsules remaining attached to form zoogloea. The individual capsules are very thick. After division the cells become separated in space by the developing capsules and are eventually arranged at an obtuse angle to each other, in a manner similar to *Aphanothece* of the blue-green algae. Swarming is preceded by a softening of the capsule and an irregular rearrangement of the cells. Individual cells separate by a slow rotatory action and once free are actively motile with polar flagella (*cf. Chromatium*). Cells are pale gray to a dirty yellow. Sulfur globules are small and confined to the peripheral layer of cytoplasm. Bacteriochlorophyll and carotenoid pigments are contained; the latter predominate to color the cell masses various shades of red or purple.

When growing in the presence of H_2S they deposit sulfur internally.

Type species: *Thiothece gelatinosa* Winogradsky.

NOTES: Only one species is described. (See also general notes, p. 48.)

Thiocystis (Thi.o.cys' tis) Winogradsky, 1888, *M p. 42.*

DIFFERENTIATING CHARACTERS: Unicellular, spherical to cylindrical organisms. They develop in small clusters, the compact clusters of 4 to 20 cells being widely separated in an almost spherical and seemingly cartilaginous capsule, the outer layer of which is neither sharply differentiated nor obviously deliquescent. As each cluster proliferates, it eventually separates into a number of smaller clusters within the zoogloeum. The 4-cell stage of development is similar in appearance to the early stages in the development of *Lamprocystis*. Single cells are rare but resemble *Chromatium*. At some stage individual clusters leave the mass either as a result of swelling and dispersion of the whole mass or a softening of the mass at one or more points. After separation, the cluster becomes motile. The process of separation of clusters observed by Winogradsky took 3 weeks. The generation time is approximately 2 days. The organisms contain bacteriochlorophyll and carotenoid pigments with the latter predominating to color the cell masses various shades of red or purple. When growing in the presence of H_2S they deposit sulfur internally.

Type species: *Thiocystis violacea* Winogradsky.

NOTES: Two species have been described, one with a diameter of 2.5 to 5.5 μ and the other less than 1 μ. (See also general notes, p. 48.)

Lamprocystis (Lam.pro.cys'tis) Schroeter, 1888. *M p. 43.* (Die Pilze Schlesiens, in Cohn, Kryptogamen-Flora von Schlesien, *3*, 1, 1886, 151.)

DIFFERENTIATING CHARACTERS: Unicellular, spherical organisms, 2.0 to 2.5 μ in diameter; capsulated. In the early stages of growth of the colony, cells form in tetr resembling *Thiocystis*. (Winogradsky c sidered that cells which form tetrads div in three directions, the result being a pointed group rather than a flat group of Further division of the cells gives rise t *compact* opaque mass of cocci embedded a large slime capsule. This is followed the formation of arched fragments, sim in appearance to sections of a hollow sphe arranged as if on the surface of a sph within the capsule. Continued growth the arched fragments results in their ed touching but not coalescing. Infolding the arched pieces takes place and fina the internal cavity is almost filled, an network resembling a sponge is produc The enveloping capsule eventually ruptu and liberates fragments of the zooglo network which reorganize into small grou interlinked by single cocci. Under un vorable conditions the cocci are vacuolat and sulfur is confined to the peripheral toplasm. Free cells are motile (*cf. Chrom tium*). The total transformation obser by Winogradsky occurred in 40 days. T organisms contain bacteriochlorophyll a carotenoid pigments, and the latter p dominate to color the cell masses vari shades of red or purple. When growing the presence of H_2S they deposit sulfur ternally.

Type species: *Lamprocystis roseopersic* (Kutzing) Schroeter.

NOTES: Only one species is described. (also general notes, p. 48.)

Amoebobacter (A.moe.bo.bac' t Winogradsky, 1888. *M p. 44.*

DIFFERENTIATING CHARACTERS: Unice lar, spherical, or rod-shaped organisms one stage of development cells are co pressed into a compact mass, colored r red, surrounded by a capsule composed an inner poorly refracting layer and outer strongly refracting layer. Placed i favorable environment the cyst cracks, a the cell mass slowly creeps out (1 to 2 da The empty cyst remains unchanged some time. The group of cells does not but grows in an irregular contoured . Whole families are motile. Most cells spherical. Division occurs only in one tion. Cells may be compressed or freely rated in the nonencysted state. There arely more than 2 to 4 within a group e colony. They are continually chang- osition and proximity in the colony. rnal colonial movement can occur with- much lateral movement. Coordinated l movement is amoeboid. Single cells sionally become isolated from the mass then are drawn back. Masses ulti- ly may separate into smaller groups. capsular material can be discerned in amilies. Families can be penetrated by l motile bacteria with ease. Wino grad- considered the connection was via ma threads but was unable to demon- e them. He considered the dense com- masses to occur in the presence of O_2 the open structure, in the presence of . Individual cells or cell masses are ap- ntly devoid of capsular material, al- gh cells within the mass may be clearly rated in space. Bacteriochlorophyll and tenoid pigments are contained with the r predominating to color the cell es various shades of red or purple. n growing in the presence of H_2S they sit sulfur internally.

ype species: *Amoebobacter roseus* Wino- sky.

TES: Three species have been de- ed, two of which are spherical and one shaped. Cell width varies from 0.5 to , depending on the species. (See also ral notes, p. 48.)

hiopolycoccus (Thi.o.po.ly.coc′ cus) ogradsky, 1888. *M p. 45.*

IFFERENTIATING CHARACTERS: Unicellu- spherical, nonmotile organisms approxi- ely 1.2 μ in diameter. Growth occurs in form of shapeless thick aggregates of ll spherical vividly colored cells. They not form hollow spherical structures. extremely smooth surface of the colony ests a limiting capsular material but bvious capsule is visible. Eventually growth, in the form of threads and flaps, appears on the surface, and cells become separated. Individual cells or cell masses are apparently devoid of capsular material, although cells within the mass may be clearly separated in space. Contain bacteriochlorophyll and carotenoid pigments, the latter predominating to color the cell masses various shades of red or purple. When growing in the presence of H_2S the organisms deposit sulfur internally.

Type species: *Thiopolycoccus ruber* Winogradsky.

NOTES: Only one species is described. (See also general notes, p. 48.)

Thiospirillum (Thi.o.spi.ril′ lum) Winogradsky, 1888. *M p. 46.*

DIFFERENTIATING CHARACTERS: Unicellular, spirally twisted* organisms, actively motile with polar flagella. The width of the cells lies within the limits of 1 to 40 μ depending on the species. Bacteriochlorophyll and carotenoid pigments are contained with the latter predominating to color the cell masses various shades of red or purple. When growing in the presence of H_2S they deposit sulfur internally.

Type species: *Thiospirillum jenense* (Ehrenberg) Winogradsky.

NOTES: Five species are described, separated on the basis of size and color of the cell mass. None has been isolated. (See also general notes, p. 48.)

Rhodothece (Rho.do.the′ ce) Molisch, 1907. *M p. 50.* (Die Purpurbakterien, Jena, 1907, 19.)

DIFFERENTIATING CHARACTERS: Unicellular, spherical organisms, 1.8 to 2.5 μ in diameter, occurring singly, in pairs, or in short chains; nonmotile. Individual cells are capsulated and usually contain two gas vacuoles (aerosomes) per cell which makes them buoyant and may give them a polygonal appearance. The organisms contain bacteriochlorophyll and carotenoid pigments; the latter predominate to color the

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* *Thiospirillum violaceum* is rarely more than a slightly bent rod and is indistinguishable from some forms of *Chromatium.*

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cell masses various shades of red or purple. When growing in the presence of H_2S they deposit sulfur internally.

Type species: *Rhodothece pendens*, Molisch.

NOTES: Only one species has been described. (See also general notes, p. 48.)

Chromatium (Chro.ma′ ti.um) Perty, 1852. *M p. 50.* (Zur Kenntniss kleinster Lebensformen, Bern, 1852, 174.)

DIFFERENTIATING CHARACTERS: Unicellular, spherical to cylindrical organisms, the latter occasionally being curved. Rod-shaped cells are of uniform width throughout. The cells occur singly and are motile with polar flagella. They may be capsulated but do not form zoogloea. They contain bacteriochlorophyll and carotenoid pigments, the latter predominating to color the cell masses various shades of red or purple. When growing in the presence of H_2S they deposit sulfur internally.

Type species: *Chromatium okenii* Perty.

NOTES: Twelve species are described, the only differentiating feature being size. Width of the cells lies within the limits of 1 to 10 μ. In view of the effect of the environmental conditions on the size of such cells, the differentiation appears invalid. Although they are described as occurring singly, they form sheets over the surface of glass containers in which they are grown, particularly on the side exposed to light. The organisms can be cultured quite readily (see method of isolation for *Chromatium*). (See also general notes, p. 48.)

Rhabdomonas (Rhab.do.mo′ nas) Cohn, 1875. *M p. 48.* (Beitr. Biol. Pflanz., *1*, Heft 3, 1875, 167.)

DIFFERENTIATING CHARACTERS: Unicellular, rod-shaped organisms of uneven width and length, often swollen, spindle-shaped, and filamentous. Cells occur singly. They are motile with polar flagella and contain bacteriochlorophyll and carotenoid pigments with the latter predominating to color the cell masses various shades of red or purple. When growing in the presence of H_2S they deposit sulfur internally.

Type species: *Rhabdomonas rosea* Col
NOTES: Three species have been
scribed. They are differentiated on
width and the presence or absence of
cium carbonate crystals in the cells. T
organisms may be variants of *Chromatiu*
The calcium carbonate may be only an
vironmental characteristic. (See also g
eral notes, p. 48.)

GREEN SULFUR BACTERIA

The following genera are all green
yellowish green and do not show the ch
acteristic absorption band for bacter
chlorophyll at 590 mμ. They contain,
far as they have been examined, *chlorobi*
chlorophyll which has an absorption ba
in the infrared region at approximately 7
mμ. They grow under strictly anaerol
conditions in the presence of sulfide a
with exposure to light. The sulfide is o
dized to sulfur which is deposited *externa*
(except with *Clathrochloris*). So far only o
of these genera, *Chlorobium*, has been is
lated.

Chlorobium (Chlo.ro′ bi.um) Nadsc
1912. *M p. 62.* (Bull. Jard. Impér. Bota
St. Pétersburg, *12*, 1912, 64 (Russian),
(German).)

DIFFERENTIATING CHARACTERS: Unicell
lar, ovoid organisms, 0.7 to 0.9 μ by 1.5
occurring singly or in chains and frequen
embedded in mucus. They do not fo
characteristic aggregates. Nonmoti
Gram-negative. Cell masses green becau
of the presence of chlorobium chlorophy
Oxidize H_2S, depositing sulfur outside t
cell.

Type species: *Chlorobium limicola* N
son.

NOTES: There are two species described
the *Manual* which are indistinguishab
morphologically. Sulfur is oxidized to s
fate by both species. *Chlorobium thios*
fatophilum Larsen will also oxidize th
sulfate, tetrathionate, and molecular
drogen.

These organisms occur in marine

water muds. Mass development of *robium limicola* occurs under conditions igh sulfide concentration and low pH.

lodictyon (Pe.lo.dic′ ty.on) Lauter- , 1913. *M p. 63.* (Allgem. botan. Z., *19*, , 98.)

FFERENTIATING CHARACTERS: Unicellu- capsulated, spherical or rod-shaped or- sms, 0.5 to 1.5 by 2.4 μ, which may egate into characteristic net-like struc- s or in two or three dimensional masses hich the cells are arranged irregularly in parallel strands. Nonmotile. Cell ses are yellowish green and contain a tosynthetic pigment which is not plant rophyll or bacteriochlorophyll. The or- isms are found in mud and stagnant er containing high concentrations of and exposed to light.

ype species: *Pelodictyon clathratiforme* fer) Lauterborn.

OTES: Three species are described in ch differentiation is based on the ar- gement of cells in the masses. Pleomor- forms appear to be common.

UTHOR'S NOTE: The difference between *aggregatum* and the mucoid growth of *orobium limicola* is not obvious from the criptions.

lathrochloris (Clath.ro.chlo′ ris) tler, 1925. *M p. 64.* (In Pascher, Die swasserflora Deutschlands, Österr. u. weiz, Jena, *12*, 1925, 457.)

IFFERENTIATING CHARACTERS: Unicellu- spherical organisms, 0.5 to 0.7 μ in di- ter, occurring in loose trellis-like ag- gates. Nonmotile. Sulfide is oxidized and ur is deposited inside the cells. Cell sses are yellowish green and contain a tosynthetic pigment.

Type species: *Clathrochloris sulphurica* afer) Geitler.

OTES: There is only one described spe- found in mud and stagnant water con- ing high concentrations of sulfide. The ure of the green pigment has not been cribed.

hlorobacterium (Chlo.ro.bac.te′ um) Lauterborn, 1915. *M p. 65.* (Verhandl. naturhist.-medizin. Ver., Heidelberg, N. F., *13*, 1915, 429.)

DIFFERENTIATING CHARACTERS: Unicellular, rod-shaped organisms, 0.5 by 2 to 5 μ, often slightly curved. Nonmotile. Found forming a covering over certain protozoan cells. Cells and cell masses are green and contain a photosynthetic pigment.

Type species: *Chlorobacterium symbioticum* Lauterborn.

NOTES: There is only one described species. Such an association between a green cell and a protozoan hardly constitutes valid grounds for the designation of a genus.

Found in pond water in Germany. No mention is made of H_2S in these ponds.

Chlorochromatium (Chlo.ro.chro.ma′ ti.um) Lauterborn, 1906. *M p. 65.* (Allgem. botan. Z., *19*, 1906, 196.)

DIFFERENTIATING CHARACTERS: Unicellular, rod-shaped organisms, 0.5 to 1.0 μ by 1 to 2.5 μ, attached in 4 to 6 rows to the surface of an actively motile, polar flagellated bacterial cell to form a barrel-shaped aggregate. The aggregate measures 2.5 to 5 μ by 7 to 12 μ. Cells are green and contain a photosynthetic pigment which is not plant chlorophyll or bacteriochlorophyll. They grow in the presence of H_2S, depositing sulfur outside the cells.

Type species: *Chlorochromatium aggregatum* Lauterborn.

NOTES: There is only one species. Unless it can be proved that the symbiosis (?) is obligatory, there appears little justification for recognition of this genus.

Found in mud and stagnant water.

Cylindrogloea (Cyl.in.dro.gloe′ a) Perfiliev, 1914. *M p. 66.* (Zhur. Mikrobiol., Epidemiol., Immunobiol., *1*, 1914, 223.)

DIFFERENTIATING CHARACTERS: Unicellular, rod-shaped organisms, 0.5 to 1.0 μ by 2 to 4 μ forming a layer on the surface of a slime capsule surrounding a colorless, cylindrical, bacteria cell. The aggregate may measure 7 to 8 μ wide and up to 50 μ long and is surrounded by another layer of slime. Nonmotile. Cells are green and contain a photosynthetic pigment which is not plant chlorophyll or bacteriochlorophyll. They grow in the presence of H_2S, depositing sulfur outside the cells.

Type species: *Cylindrogloea bacterifera* Perfiliev.

NOTES: There is only the one species. Perfiliev emphasizes that the species designation is only a provisional one.

Found in mud and stagnant water.

RED, PURPLE, AND BROWN NON-SULFUR BACTERIA

The following genera contain bacteriochlorophyll and carotenoid pigments. The former gives an absorption band at 590 mμ in the intact cells and 774 mμ in alcoholic extracts.

They are all capable of growth anaerobically in the presence of suitable hydrogen donors and when *exposed to light*. Some species grow in the dark but only aerobically.

They do not oxidize H_2S (SH^-) and therefore do not deposit sulfur internally or externally.

Rhodopseudomonas (Rho.do.pseu.do.mo′ nas) Kluyver and van Niel, 1937, and van Niel, 1944. *M p. 53.* (Kluyver and van Niel, in Czurda and Maresch, Arch. Mikrobiol. *8*, 1937, 119; and van Niel, Bacteriol. Revs., *8*, 1944, 86.)

DIFFERENTIATING CHARACTERS: Unicellular, rod-shaped or spherical organisms less than 2 μ wide; motile with polar flagella. They do not aggregate in the form of radial colonies. Gram-negative. They contain bacteriochlorophyll and carotenoid pigments, the latter predominating. They do not oxidize H_2S.

Type species: *Rhodopseudomonas palustris* (Molisch) van Niel.

NOTES: Four species, *R. palustris*, *R. gelatinosa*, *R. capsulata*, and *R. spheroides* have been described in the *Manual.*

R. spheroides is predominantly spherical in all media.

R. capsulata is rod-shaped in media above pH 7.0 and spherical in more acid media. The other species are rod-shaped. All species show a considerable degree of pleom phism with the shape varying with the and the substrate. Pleomorphic forms frequently nonmotile.

The width of cells varies from 0.5 μ to 4 μ (*R. spheroides*). Capsulated for are common. Growth occurs over a range of 6.0 to 8.5.

The color of the colonies varies with conditions of growth and is affected light, oxygen, and the nature of the c bon substrate. Anaerobic cultures of *capsulata, R. spheroides,* and *R. palustris* yellowish brown, greenish brown, or re dish-brown. All three of these species c be adapted to aerobic growth when colon are distinctly red. Most cultures of *gelatinosa* will not grow aerobically. Anae bic growth is pinkish.

Species other than *R. capsulata* may p duce a water-soluble bluish red pigme which is not a carotenoid.

Although capable of photosynthetic m tabolism, the species are not autotroph being dependent on growth factors a usually on organic hydrogen donors.

Differentiation is based on morphologic and nutritional characteristics.

(See also methods of isolation)

Rhodospirillum (Rho.do.spi.ril′ lu Molisch, 1907, emend. van Niel 1944. *p. 58.* (Molisch, Die Purpurbakterie Jena, 1907, 24; van Niel, Bacteriol. Re *8*, 1944, 86.)

DIFFERENTIATING CHARACTERS: Unicel lar, spiral-shaped organisms less than 2 wide; motile with polar flagella. Gra negative. Contain bacteriochlorophyll a carotenoid pigments, the latter predo nating. They do not oxidize H_2S.

Type species: *Rhodospirillum rubr* (Esmarch) Molisch.

NOTES: Four species have been describe *R. rubrum, R. fulvum, R. molischianum,* a *R. photometricum.*

The depth and amplitude of the spir vary with the species and conditions growth. Half spirals are frequently pr duced. A cyclic development similar to th ribed by Williams and Rittenberg ern. Bull. Bacteriol. Nomen. and on., *7*, 1957) for the genus *Spirillum* not yet been reported for the genus *lospirillum*.

e colonies of *R. rubrum* are a deep red. ddition to the band at 590 mμ for the eriochlorophyll, this species shows an- r band at 550 mμ for spirilloxanthin h is absent from the other species h have bands at 520 mμ and 485 mμ carotenoids. The colonies of other spe- are reddish brown to orange.

elatin is not liquefied.

hiosulfate is not oxidized.

xcept for *R. fulvum*, glucose and other ohydrates and polyalcohols are *not* able substrates.

ll species oxidize ethanol and certain y and hydroxy acids and amino acids.

one of the species is capable of strictly otrophic growth.

hey are all strict anaerobes with the eption of *R. rubrum*.

annielia (Van.ni.el′i.a or Van. l′i.a) Pringsheim, 1955. (J. Gen. Mi- biol., *13*, 1955, 285.)

his genus does not appear in the enth edition of the *Manual*. It has re- tly been described as a pink polar flagel- which, in natural media, aggregates small radial colonies. Pringsheim ed, however, to obtain such colonies in tone media. This is possibly another cies of *Rhodopseudomonas*.

ype species: *Vannielia aggregata* Pring- im.

Rhodomicrobium (Rho.do.mi.cro′bi.um) Duchow and Douglas, 1949. *M p. 277.* (J. Bacteriol., *58*, 1949, 409.)

DIFFERENTIATING CHARACTERS: Oval to rod-shaped cells which reproduce by producing a long thin (0.1 to 0.2 μ) tubular process from which a new cell is produced. The tubular process may branch. Individual cells are motile with polar flagella. Gram-negative. They contain bacteriochlorophyll and carotenoid pigments with the latter predominating. They do not oxidize H_2S.

Type species: *Rhodomicrobium vannielii* Duchow and Douglas.

NOTES: Only one species has been recorded. The mature cells are ovoid and measure 1.2 by 2.8 μ. One to three daughter cells may be produced by branching of the tubular outgrowths. A thickening appears in the tube between the mother and daughter cell.

Colonies are a dark orange-red.

Growth occurs anaerobically in the light and in the presence of a suitable organic hydrogen donor such as ethanol, propanol, butanol, acetate, propionate, butyrate, valerate, cuproate, lactate, and malate.

Glucose, mannose, fructose, mannitol, citrate, tartrate, formate, thiosulfate, and sulfide are not utilized.

The optimal temperature is 25 to 31° C.

The genus *Rhodomicrobium* has a counterpart in the colorless *Hyphomicrobium*. (It should be noted that Griffiths and Stanier, (J. Gen. Microbiol., *14*, 1956, 698) have produced colorless, nonphotosynthetic variants of *Rhodopseudomonas* by exposure to ultraviolet light).

NONPHOTOSYNTHETIC ORGANISMS

Hyphomicrobium (Hy.pho.mi·cro′ um) Stutzer and Hartleb, 1898. *M 277.* (Mitt. Landwirtsch. Inst. d. k. iv. Breslau, 1898.)

DIFFERENTIATING CHARACTERS: Colorless, spherical to ovoid organisms, 0.5 by 1.0 μ when mature; motile with a single polar flagellum; reproduce by the production of a tubular outgrowth, 0.2 to 0.3 μ wide, on the end of which a daughter cell is formed. The tubular outgrowths may be simple or branched. Daughter cells are initially spherical but later become ovoid.

Type species: *Hyphomicrobium vulgare* Stutzer and Hartleb, 1898.

NOTES: A single species is described. The predominant growth habit is a dense group of cells from which filaments radiate outwards.

The organism grows well on a formate-nitrate agar but poorly on gelatin or peptone agar.

Formate, formaldehyde, methanol, acetate, and lactate are suitable carbon sources.

Aerobic; optimal temperature 25° C.

Found in soil.

Nitrosomonas (Ni.tro.so.mon′ as) Winogradsky, 1890. *M p. 68.* (Ann. inst. Pasteur, *4*, 1890, 257.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, ovoid to rod-shaped organisms less than 2 μ wide; motile with a single polar flagellum; not capsulated; Gram-negative; *aerobic*; do not oxidize ferrous iron or deposit iron oxides in or on the cells; do not grow on meat extract agar or other complex organic media; strict autotrophs which use carbon dioxide as the sole source of carbon and derive their energy from the oxidation of ammonia to nitrite.

Type species: *Nitrosomonas europea* (Winogradsky.

NOTES: Two species have been described (*N. europea* and *N. monocella*). The optimal pH is approximately 8.0 to 9.0 with little or no growth below pH 7.0.

Found in soils.

Nitrosococcus (Ni.tro.so.coc′ cus) Winogradsky, 1892. *M p. 69.* (Arch. sci. biol., St. Pétersburg, *1*, 1892, 127.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, spherical organisms less than 2 μ in diameter; Gram-positive; *aerobic*; do not deposit oxides of iron in or on the cells; strict autotrophs which will not grow in organic media but use carbon dioxide as the sole source of carbon and obtain their energy from the oxida of ammonia to nitrite.

Type species: *Nitrosococcus nitrosus* (gula) Buchanan.

NOTES: Only one species is descri Cells measure 1.5 to 1.7 μ in diameter are surrounded by a thick cell membr Motility has not been demonstrated. S turbid yellowish colonies are produced silica gel.

Nitrosospira (Ni.tro.so.spi′ ra) S. nogradsky, 1931. *M p. 70.* (Compt. re *192*, 1931, 1004.)

DIFFERENTIATING CHARACTERS: Free- ing, rigid, unicellular, colorless, spi shaped organisms less than 2 μ wide; *a bic*; autotrophic with carbon dioxide u as the sole source of carbon and energy tained by the oxidation of ammonia nitrite.

Type species: *Nitrosospira briensis* Winogradsky and H. Winogradsky, 1933

NOTES: Two species are described. both species the spirals are very clos wound and may be up to 20 μ long. M tility is not recorded but is presumably means of flagella. Optimal pH 7.0 to 7.2

Found in soil.

Nitrosocystis (Ni.tro.so.cys′ tis) Winogradsky, 1931. *M p. 70.* (Com rend., *192*, 1931, 1003.)

DIFFERENTIATING CHARACTERS: Free- ing, unicellular, colorless, rod-shaped ganisms less than 2 μ wide; capsulated a aggregated in zoogloea which become cysted; motile with polar flagella or n motile; *aerobic*; strict autotrophs which not grow on meat infusion agar or ot complex organic media; carbon diox used as the sole source of carbon and ergy obtained from the oxidation of a monia to nitrite; do not oxidize ferrous ir or deposit iron oxide in or on the cells.

Type species: *Nitrosocystis javanen* (Winogradsky) Starkey.

NOTES: Two species are described. M tility is not described for one species (*coccoides*).

Winogradsky differentiated *Nitroso*

rom *Nitrosocystis* on the formation of
(or clear) colonies by the former and
(or dark) colonies by the latter. Con-
able doubt has been cast on the va-
of this differentiation by Meikeljohn
ure, *16*, 1951, 56, and J. Soil Sci., *4*,
, 62) who states that the nature of the
ies is largely determined by conditions
lture. Kingma Boltjes (Arch. Mikro-
, *6*, 1935, 69) claims to have obtained
types from single cell isolates of *Nitro-*
mas. Imsenecki (Nature, *157*, 1946,
and Grace (Nature, *168*, 1951, 117)
est that the organisms are myxobac-
or cultures are contaminated with
obacteria.

oth species were isolated from soils.

itrosogloea (Ni.tro.so.gloe′ a) H.
ogradsky, 1935. *M p. 71.* (Compt.
l., *200*, 1935, 1887.)

FFERENTIATING CHARACTERS: Free-liv-
unicellular, colorless, coccoid to rod-
ed organisms less than 2 μ wide; cap-
ted and aggregated into zoogloeae
h do not become encysted; *aerobic*;
t autotrophs which will not grow on
t infusion agar or other complex organic
lia; use carbon dioxide as the sole source
arbon and obtain their energy by the
ation of ammonia to nitrite. Do not
ize ferrous iron or deposit iron oxides
r on the cells.

ype species: *Nitrosogloea merismoides*
Winogradsky.

OTES: Three species are recorded, dif-
ntiated apparently on morphology and
nial characters. Motility is not stated.
s may be arranged in chains or at ran-
n in the zoogloea.

hese may also be only a growth form of
rosomonas.

itrobacter (Ni.tro.bac′ ter) Wino-
dsky, 1892. *M p. 72.* (Arch. sci. biol.,
Pétersburg, *1*, 1892, 127.)

IFFERENTIATING CHARACTERS: Free-liv-
, unicellular, colorless, rod-shaped or-
isms less than 2 μ wide; do not form
gloea; motile by means of a single polar
ellum or nonmotile; *aerobic*; strict autotrophs which do not grow on meat infusion agar or other complex organic media; use carbon dioxide as the sole source of carbon and obtain their energy by the oxidation of nitrite to nitrate. Do not oxidize ferrous iron or deposit oxides of iron on or in the cells.

Type species: *Nitrobacter winogradskyi* Winslow *et al.*

NOTES: Two species are described. The Gram stain is variable. On silica gel or washed agar media the colonies are minute and take 7 to 10 days to develop. They are, on the surface of media, frequently only one cell deep and are clear to slightly brownish. Growth is inhibited by organic substances (particularly of a nitrogenous nature) and by ammonium ions in an alkaline medium (*N. winogradskyi*). Limits of growth pH 6.6 to 10.0 (*N. agilis*). Optimal temperature range 25° to 28° C.

Found in soils.

Nitrocystis (Ni.tro.cys′ tis) H. Winogradsky, 1935. *M p. 73.* (Trans. Third Cong. Intern. Soil Sci., Oxford, *1*, 1935, 139.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, rod-shaped organisms less than 2 μ wide; capsulated and aggregated into zoogloea; motility is not recorded; *aerobic*; strict autotrophs which will not grow on meat infusion agar or other complex organic media; use carbon dioxide as the sole source of carbon and obtain their energy from the oxidation of nitrite to nitrate; do not oxidize ferrous iron or deposit oxides of iron on or in the cells.

Type species: *Nitrocystis sarcinoides* H. Winogradsky.

NOTES: Two species are recorded. Cells of the type species are grouped in sarcina-like packets. Colonies on silica gel are amber to yellow and wart-like. Old colonies shrink to hard sand-like grains. Both species have been isolated from activated sludge. The strictly autotrophic nature stated above is presumed. No mention of it is made in the *Manual.*

Methanomonas (Me.tha.no.mo′nas) Orla-Jensen, 1909. *M p. 74.* (Zentr. Bakteriol. Parasitenk., Abt. II, *22*, 1909, 311.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, rod-shaped organisms, 0.6 by 1.0 μ; motile with a polar flagellum; Gram-negative; *aerobic*; grow autotrophically in a mineral salts medium with ammonia nitrogen and under an atmosphere containing methane, oxygen, and carbon dioxide, oxidizing the methane to carbon dioxide and water. Methane and methanol the only carbon compounds used.

Type species: *Methanomonas methanica* (Sohngen) Orla-Jensen.

NOTES: The above information is taken from a paper by Dworkin and Foster, (J. Bacteriol., *72*, 1956, 646). According to these authors, Sohngen's isolates used a number of organic substrates all of which failed to support growth of their isolates. Foster* points out, however, that the claims made for growth factor requirements in the paper cited above are invalid. The organisms will grow in a purely mineral medium. See Methods, p 151.

The optimal gas phase is one containing methane, 10 to 90 per cent; oxygen, 15 per cent; and CO_2, 0.3 per cent.

The organism produces a pink pellicle on liquid media. The pigment is insoluble in water, soluble in *n*-hexane, chloroform, and ether. The absorption spectrum of a chloroform extract has a clear maximum at 503 mμ and has properties of carotenoids.

Hydrogenomonas (Hy.dro.ge.no.mon′as) Orla-Jensen, 1909. *M p. 75.* (Zentr. Bakteriol. Parasitenk., Abt. II, *22*, 1909, 311.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, straight, rod-shaped organisms less than 2 μ in width; colorless or yellowish but not photosynthetic; motile by means of polar flagella or nonmotile; *aerobic*; Gram-negative; facultative autotrophs which grow readily on organic media but are able to grow in a mineral medium with carbon dioxide as the sole sourc carbon and energy obtained by oxidi hydrogen to water.

They *do not or are not known to* hydro agar, cellulose, chitin, or alginates; proc acids from carbohydrates; utilize phe oxidize thiosulfate autotrophically; oxi ethyl alcohol quantitatively to acetic a produce alcohol from glucose; or fix atr pheric nitrogen. They are not haloph

Type species: *Hydrogenomonas pa tropha* (Kaserer, 1906) Orla-Jensen, 1

NOTES: Four species are described in *Manual.* Rods are 0.3 to 0.5 μ by 1 to Two of the four species are recordec Gram-negative. Two species are capsula Colonies on inorganic media are butyr or mucoid, depending on the species, are colorless (1 species) or slightly yell ish (3). In liquid mineral salts media p cles are formed by three of the four spec

Hydrogen is oxidized but CO_2 is not fi when organic hydrogen donors have b used in respiration experiments with *facilis.* There are numerous bacteria wh can oxidize hydrogen in respiratory exp ments but are incapable of autotrop growth at the expense of H_2 and CO_2 mineral media. Growth and not hydro oxidation is therefore the cardinal char teristic.

The two species which have been scribed more fully have failed to prod acid from carbohydrates, are indole-ne tive and H_2S-negative, and produce sli alkalinity in litmus milk.

Lack of acid formation in carbohydra and production of alkalinity in prot media are general characteristics of polar flagellate heterotrophs. There are ceptions.

Found in soil and water. Optimal te perature, 28° C. The optimal gas ratio growth is approximately 10 per cent C 30 per cent air, and 60 per cent hydrog

Carboxydomonas (Car.box.y.d mon′as) Orla-Jensen, 1909. *M p.* (Zentr. Bakteriol. Parasitenk., Abt. *22*, 1909, 311.)

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* Personal communication.

FERENTIATING CHARACTERS: Free-liv- unicellular, colorless, straight, rod- ed organisms less than 2 μ wide; non- e; capable of growth in a mineral salts um with *carbon monoxide* used as the source of carbon.

pe species: *Carboxydomonas oligocar- ilus* (Beijerinck and van Delden, 1903) Jensen, 1909.

TES: There is considerable doubt about dentity of this organism. Kistner (Ko- l. Ned. Akad. Wetenschap., Proc. Ser. 6, 1953, No. 4) has shown that species *Hydrogenomonas* can oxidize carbon oxide. (For more detailed comments the *Manual*).

hiobacterium (Thi.o.bac.te′ri.um) e, 1924. *M p. 79.* (Allgem. Tech. robiol., Teil I, 1924, 68.)

FFERENTIATING CHARACTERS: Unicellu- rod-shaped organisms, 0.3 to 1 5 μ by 5 μ which grow in colonies on the sur- of water containing sulfide and deposit r either inside or outside the cells. species produces a bladder-like colony cells embedded in the surface. Motil- has not been observed.

ype species: *Thiobacterium bovista*, e.

TES: Three species have been de- ed. Colonies floating on water are e by reflected light and black by trans- ed light.

THOR'S NOTE: These organisms need r examination. Oxidation of sulfide at water surface may account for the ap- ance of sulfur in the colonies but not e cells.

acromonas (Mac.ro.mo′nas) Uter- l and Koppe, 1923. *M p. 80.* (Verhandl. ern. Ver. f. Theoret, u. angew. Limno- e, 1923, 86.)

IFFERENTIATING CHARACTERS: Large, ellular, cylindrical pear-shaped or htly curved rods, 3 to 14 μ wide. Ac- ly motile with a single polar flagellum. y contain large spherules of calcium onate and possibly oxidize sulfide, de- ting sulfur inside the cells.

The presence of $CaCO_3$ may be dependent upon an environment rich in Ca^{++}.

Type species: *Macromonas mobilis* (Lauterborn) Utermöhl and Koppe.

NOTES: Two species are described which differ mainly in size. Found in a fresh water environment containing sulfide and calcium.

Thiovulum (Thi.o′vu.lum) Hinze, 1913 *M p. 81.* (Ber. deut. botan. Ges., *31*, 1913, 195.)

DIFFERENTIATING CHARACTERS: Unicellular, spherical to ovoid organisms, 5 to 20 μ in diameter, with the cytoplasm compressed in one end of the cell. Sulfur is deposited in the cytoplasm. The cells have an extremely rapid darting motion in liquids suggestive of flagella but these have never been demonstrated. They are found in sea water over decomposing organic matter liberating H_2S, where growth occurs in the form of a tenacious web in a zone of critical H_2S-O_2 concentration. Not yet isolated but easily recognized.

Type species: *Thiovulum majus* Hinze.

NOTE: There is only one described species. Enrichment cultures have been obtained.

A recent paper by E. Fauré-Fremiet and C. Rouiller (Exptl. Cell Research, *14*, 1958, 29) has given some excellent micrographs of sections of *Thiovulum* which tend to confirm its bacterial nature, but no flagella could be found. Instead, a peculiar fibrillar polar apparatus has been discovered; its true function is not understood, but a motor function is suggested.

Thiospira (Thi.o.spi′ra) Vislouch, 1914. *M p. 82.* (J. Microbiologie, *1*, 1914, 50.)

DIFFERENTIATING CHARACTERS: Rigid, unicellular, curved organisms, 6 to 50 μ long, actively motile with a polar flagellum. Sulfur is deposited inside the cells.

Type species: *Thiospira winogradskyi* (Omelianski) Vislouch.

NOTES: Two species are described. They differ in size and distribution of the sulfur granules. Although the name suggests a spiral axis there is little evidence of this in the descriptions or illustrations which suggest only a curvature in the rods. They are probably anaerobic.

Found in curative muds and sea water.

Thiobacillus (Thi.o.ba.cil′lus) Beijerinck, 1904. *M p. 83.* (Zentr. Bakteriol. Parasitenk., Abt. II, 11, 1904, 593.)

DIFFERENTIATING CHARACTERS: The genus is divisible into two groups, the strict autotrophs and facultative autotrophs.

The *strict autotrophs* are free-living, unicellular, colorless, straight, rod-shaped organisms; motile with polar flagella or nonmotile; Gram-negative; *aerobic*; will not grow on meat extract agar or other complex organic media; carbon dioxide used as the sole source of carbon and their energy is obtained by the oxidation of thiosulfate.

The *facultative autotrophs* differ from the strict autotrophs in their ability to grow on organic media.

Type species: *Thiobacillus thioparus* Beijerinck.

NOTES: Nine species are recorded. The Gram stain is not recorded for two species. Cells measure 0.5 by 1 to 3 μ except *T. coprolyticus* (which measures 0.1 to 0.2 μ by 6 to 8 μ); some species are motile with polar flagella, others are nonmotile.

Five species are acknowledged as strict autotrophs; three are facultative. The facultative autotrophs give a moderate to copious growth on nutrient agar. No acid production from carbohydrates is reported.

All species oxidize thiosulfate with sulfate as one of the end products and can utilize CO_2 as the sole source of carbon. Species differentiation is based on the nature of sulfur compounds oxidized and the products of thiosulfate oxidation.

Those species which oxidize S^0 are all strict autotrophs, and the end product is only sulfuric acid. The oxidation of S^0 is associated with a fall in pH, the extent of which is determined by the pH tolerance of the cells.

H_2S is oxidized by several but not all species. Those which do, will tolerate 200 p.p.m. H_2S mixed with air. One per cent H_2S in air is toxic. The H_2S is oxidize
sulfuric acid with an associated fall in

Pellicle formation in liquid thiosul
media is usually associated with the
duction of sulfur.

Oxidation of thiosulfate follows four
ferent pathways either as one- or two-
reactions.

A. $6\ Na_2S_2O_3 + 5\ O_2 \rightarrow$

$4\ Na_2SO_4 + 2\ Na_2S$

$2\ Na_2S_4O_6 + 6\ H_2O + 7\ O_2 \rightarrow$

$2\ Na_2SO_4 + 6\ H_2S$

Tetrathionate is formed and reoxidi
Curiously, two of the organisms which
this, *T. concretivorus* and *T. thiooxida*
cannot oxidize tetrathionate alone. *T. n*
politanus will oxidize the tetrathionate.
There is a fall in pH

B. $5\ Na_2S_2O_3 + H_2O + 4\ O_2 \rightarrow$

$5\ Na_2SO_4 + H_2SO_4 +$

$2\ S + 3\ O_2 + 2\ H_2O \rightarrow 2\ H_2SO_4$

brought about by *T. thioparus*. There i
fall in pH

C. $Na_2S_2O_3 + 2\ O_2 + H_2O \rightarrow$

$Na_2SO_4 + H_2S$

brought about by *T. novellus*—a facult
tive autotroph. There is a fall in pH

D. $2\ Na_2S_2O_3 + H_2O + \frac{1}{2}\ O_2 \rightarrow$

$Na_2S_4O_6 + 2\ Na$

brought about by some facultative au
trophs. There is a rise in pH someti
followed by a slight fall to the origi
value.

Ammonia nitrogen is used by all orga
isms. Nitrate nitrogen is either used, n
used, or is toxic. NO_3 is reduced to N_2 b
T. denitrificans.

Colonies on silica gel or washed thios
fate agar are small and colorless or, wi
species precipitating S^0 from the oxidati
of thiosulfate, they may be yellowish
white.

ferrooxidans on media containing fer-
iron becomes dark brown from the
ation of the ferrous to ferric iron at
pH. Other species do not deposit iron
t the cells.

seudomonas (Pseu.do′ mo.nas or
.do.mo′ nas) Migula, 1894. *M p. 89.*
). bakteriol. Inst. Karlsruhe, *1*, 1894,
)

FFERENTIATING CHARACTERS: Free-
g, unicellular, nonphotosynthetic, non-
ing, straight, rod-shaped organisms less
2 μ wide; arranged singly; motile with
r flagella or nonmotile (rare); Gram-
tive; *aerobic*; heterotrophic organisms
ch do not deposit iron in or on the cells.
hey *do not or are not known to*: fix at-
pheric nitrogen, oxidize ethyl alcohol
acetic acid, oxidize thiosulfate auto-
hically, produce alcohol from glucose
netabolize alkylamines.

ype species: *Pseudomonas aeruginosa*
hroeter) Migula.

OTES: Although the genus *Pseudomonas*
oys the same ranking as other genera of
ar flagellates in the *Manual*, it is the
hor's opinion that it represents only
t group of species which remains after
separation of the other genera, based
specific physiological properties, from
large group of polar flagellates collec-
ly placed in the Order *Pseudomonadales.*

n the *Manual* 149 species are described,
t of which are ovoid to short rods,
tile with a single polar flagellum or hav-
lophotrichous flagella. Only six species
recorded as nonmotile.

he majority of species will grow in a
eral salts medium with ammoniacal
rogen and a simple carbon source. Only
ew require aminonitrogen or growth fac-
s (*e.g., P. pseudomallei*). They usually
w well on peptone media, producing
ire, translucent, butyrous low convex to
bonate colonies 1 to 3 mm. in diameter
24 hours.

A large number of species produce water-
uble and sometimes fluorescent pigments
(*e.g.*, pyocyanin, fluorescin, chlororaphine) which diffuse into the medium and leave the colonies an off-white in color. Pigmentation is not a constant feature and varies with the medium. *P. iodinum* produces a purple pigment whereas *P. indoloxidans* produces blue crystals of indigotin from indole. Soluble red pigments are occasionally produced.

Pyocyanin is a "redox" indicator which becomes colorless in the reduced state. In liquid media only the upper layer may appear bluish green. On shaking, the dye reoxidizes.

The genus is essentially oxidative. Species examined all oxidize glucose to gluconic acid, 2-ketogluconic acid, and other derivatives. In liquid media the limited oxygen supply results in a slow acid production which in peptone media may be obscured by a more rapid production of ammonia.

Among substances attacked are cellulose (by six species), agar (by one), hexoses, pentoses, mono-, di-, and tricarboxylic acids, hydroxy acids, and various hydrocarbons including kerosene and gasoline. Lactose is rarely attacked (27/132).* Gas is not produced except by *P. petasitis.*

The majority actively attack amino acids, peptones, and some proteins, particularly gelatin which is hydrolyzed by 99/148 species. Coagulated blood serum is liquefied by 9/11 species.

Indole is produced by 24/125.

Starch is hydrolyzed by 36/91.

Fats are hydrolyzed by 15/35.

Marine species require about 3 per cent added salt but are not strongly halophilic.

Nitrite is produced from nitrate by 60/127 and nitrogen by 5/127.

The organisms are essentially oxygen-dependent although this is not obligatory. Alternative hydrogen acceptors such a nitrate and malate are reduced by some species in the absence of oxygen, the former

* The number of species positive over the number tested. See Introduction, p. vii, for further explanation.

giving rise via nitrite to ammonia, nitrous oxide, or nitrogen gas.

At least half the species in the genus and possibly all of them are found in soil or water. Ninety of the 149 recorded species are pathogenic to plants. Two species *P. aeruginosa* and *P. pseudomallei* are animal pathogens. *P. aeruginosa* is also a plant pathogen and occurs in water and soils.

The heterogenous nature of the genus is indicated by no fewer than 22 entries in the key.

Xanthomonas (Xan.tho′mo.nas or Xan.tho.mo′nas) Dowson, 1939. *M p.* 152. (Zentr. Bakteriol. Parasitenk., Abt. II, *100*, 1939, 187.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, yellow-pigmented, nonphotosynthetic, nonsporing, straight rods less than 2 μ wide; arranged singly; motile with polar flagella; heterotrophic organisms; *aerobic*; Gram-negative; pathogenic to plants causing necrotic infections.

They *do not or are not known to*: produce alcohol from glucose, oxidize ethyl alcohol quantitatively to acetic acid, fix atmospheric nitrogen, or deposit iron in or on the cells.

Type species: *Xanthomonas hyacynthi* (Wakker) Dowson.

NOTES: Sixty species are described. They are all Gram-negative rods, usually 1 to 3 μ long.

The majority of species grow well on ordinary nutrient agar and produce colonies which have a nondiffusible pigment which is some shade of yellow. Starr* has suggested that the pigment is specific xanthophyll pigment which may prove characteristic of the group. Colonies are usually opaque.

Fifty-five out of 58 tested species liquefy gelatin, and 52/60 cause an alkaline peptonization of milk. Six are reported to liquefy coagulated serum.

Thirty-seven out of 50 species tested produce acid from glucose and lactose and
frequently from other carbohydrates. Th
species produce gas.

Nitrates are reduced to nitrite by 14/
and 8/46 produce indole.

H_2S is produced by 44/50.

Starch is hydrolyzed by 48/51.

Of 25 species 19 are lipolytic, and 15
of 26 cause a liquefaction of pectate me

Acetobacter (A.ce.to.bac′ter) B
jerinck, 1898. *M p. 183.* (Quoted fr
Kral's Sammlung v. Mikroorg., Prag
1898, 7.)

DIFFERENTIATING CHARACTERS: Fr
living, unicellular, nonsporing, nonpho
synthetic, straight, rod-shaped organis
less than 2 μ wide; motile with flagella
nonmotile; Gram-negative; *aerobic*; hete
trophic organisms which do not depo
iron in or on the cells; oxidize ethyl al
hol quantitatively to acetic acid.

Type species: *Acetobacter aceti* (Bei
rinck) Beijerinck.

NOTES: Seven species are described. Th
measure on the average 0.5 to 1.0 μ by 1
2 μ. The type of flagellation is genera
stated as polar although Leifson (Anto
van Leeuwenhoek., J. Microbiol. Ser
20, 1954, 102) disputes this, stating th
the species which oxidize acetic acid ha
peritrichous flagella.

This has been confirmed by Shimw
(Antonie van Leeuwenhoek., J. Microbi
Serol., 1958, *24*, 187) who has also co
firmed Leifson's observation that pe
trichous flagellation is confined only to t
acetate-oxidizing group. He strongly su
ports the division of the genus *Acetobac*
into the two genera *Acetomonas* Leifs
and *Acetobacter* Beijerinck as proposed
Leifson, the former to be broadened to
clude all the polar flagellated species a
the latter the peritrichously flagellated a
also nonmotile species which oxidize ac
tate.

Under optimal conditions the rods are
uniform size and shape. Under abnorm
conditions of medium or temperatu
bizarre pleomorphic forms are produced.

Occur singly, in chains, or in zooglo

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* Personal communication.

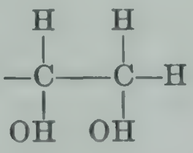
es. Some species are capsulated. *A.* *um* produces a capsule of pure cellu-

ne Gram stain is recorded for only one es, although the genus is generally idered as Gram-negative.

owth is poor or absent on peptone ia in the absence of carbohydrates or nols. The best growth is obtained on t extract media containing glucose or ort media. Colonies are gray to a buff n and may be butyrous, mucoid, or laginous. *A. melanogenum* produces a k pigment and *A. roseum* a red one in able media. In liquid media pellicles common.

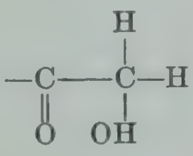
he organisms all oxidize primary alco- to the corresponding acids. The oxida- of ethyl alcohol to acetic acid in quan-s ranging from 2 to 10 per cent gives genus its name. Many other organisms g about a similar oxidation but in small unts only. The latter rarely tolerate strongly acidic environment in which species of *Acetobacter* grow.

he oxidation of secondary alcohols de-ds on the relative position of the α and –OH groups. If they are on the same of the chain



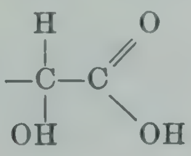
$$\begin{array}{ccc} \text{H} & & \text{H} \\ | & & | \\ -\text{C} & — & \text{C}-\text{H} \\ | & & | \\ \text{OH} & & \text{OH} \end{array}$$

e species (the ketogenic group) oxidize β—OH group to a ketone

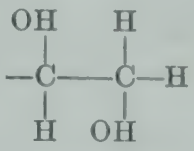


$$\begin{array}{ccc} & & \text{H} \\ & & | \\ -\text{C} & — & \text{C}-\text{H} \\ \| & & | \\ \text{O} & & \text{OH} \end{array}$$

ereas the others (the aketogenic group) dize the α—OH to the acid

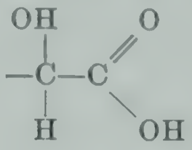


When the two —OH groups are on opposite sides



$$\begin{array}{ccc} \text{OH} & & \text{H} \\ | & & | \\ -\text{C} & — & \text{C}-\text{H} \\ | & & | \\ \text{H} & & \text{OH} \end{array}$$

they all oxidize the α group to the acid



The majority of species produce acid from glucose, ethyl alcohol, propyl alcohol, and glycol. Beyond this the range of substances oxidized is limited. Lactose is not attacked by any species.

Acid production is by oxidation, even with glucose, the latter being oxidized to gluconic or 5-ketogluconic acid.

A suggested difference between the genus *Acetobacter* and related genus *Pseudomonas* is that the former oxidizes maltose to gluconic and 5-ketogluconic acid whereas species of *Pseudomonas* produce maltobionic acid. This needs further study, particularly in view of the findings of Leifson and o Shimwell.

The optimal temperature is 28° to 30° C for most species.

The organisms are usually found in fer menting liquids.

Aeromonas (A.e.ro.mo′ nas) Kluyve and van Niel, 1936. *M p. 189.* (Zentr. Bakteriol. Parasitenk., Abt. II, *94*, 1936, 398.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, straight rod-shaped organisms, less than 2 μ wide, occurring singly or in pairs; motile with polar flagella or nonmotile; Gram-negative; *aerobic*; heterotrophic; liquefy gelatin; acidify, coagulate, and peptonize milk; reduce nitrates to nitrite and produce acid and gas from glucose. Pathogenic to fish, eels, and frogs.

They *do not or are not known to*: deposit iron in or on the cells, hydrolyze cellulose or agar, utilize phenol, oxidize ethyl alcohol quantitatively to acetic acid, oxidize thiosulfate autotrophically, produce ethyl alcohol from glucose or fix atmospheric nitrogen. They are not bioluminescent.

Type species: *Aeromonas liquefaciens* (Beijerinck) Kluyver and van Niel.

NOTES: Four species are described. They are rods 0.5 to 1.0 μ by 1 to 3 μ, not capsulated. Three of the four species are motile with polar flagella.

The organisms grow well on nutrient agar. The ability to grow in a mineral medium with ammonium salts as the sole source of nitrogen is not recorded. *A. salmonicida* requires methionine and arginine.

Colonies on nutrient agar are 1 to 2 mm. in diameter in 24 hours, circular, entire, and translucent.

H_2S and indole are produced and starch is hydrolyzed by three of the four species.

Acid is produced from lactose by one species.

The organisms occur in fresh and polluted water and are pathogenic for fish, eels, and frogs. They cause a fatal bacteremia in some laboratory animals. The optimal temperature varies with the species.

Some bioluminescent organisms with essentially the same characteristics have been placed in the genus *Photobacterium*.

Photobacterium (Pho.to.bac.te′ri.um) Beijerinck, 1889, emend. Breed and Lessel, 1954. *M p. 193.* (Beijerinck, Arch. néerl. sci., *23*, 1889, 401; Breed and Lessel, Antonie van Leeuwenhoek. J. Microbiol. Serol., *20*, 1954, 60.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing organisms less than 2 μ in width; Gram-negative; *aerobic*; heterotrophic; *bioluminescent* on media made from sea water or containing 3 per cent salt.

They *do not or are not known to:* hydrolyze cellulose, chitin or agar, utilize phenol, oxidize thiosulfates autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce alcohol from glucose in appreciable amounts, or fix atmosph nitrogen.

Type species: *Photobacterium phos reum* (Cohn) Ford.

NOTES: Four species are described. T may be coccoid or straight or curved r Rudimentary branching may occur in paragine media. Motile species have p flagella.

Despite the lengthy descriptions of spe given in the *Manual*, there has been insu cient uniformity in the method of desc tion to merit enumeration of reactions, there is little other than bioluminesce upon which to base a general descripti See Spencer (J. Gen. Microbiol. *13*, 19 111) for discussion on the classification the luminescent bacteria.

The organisms are found on fresh a decaying fish. One species was isola from the photogenic organ of the Cep lopod *Rondeletia minor*.

Azotomonas (A.zo.to.mo′nas) Sta 1940. *M p. 198.* (Zentr. Bakteriol. Pa sitenk., Abt. II, *102*, 1940, 18.)

DIFFERENTIATING CHARACTERS: Free-l ing, unicellular, coloress, straight, n poring, rod-shaped organisms less than in width; motile with polar flagella; hete trophic; *fix atmospheric nitrogen when gro ing in a mineral salts medium with a su able carbon source.* Gram-negative. *Aerob*

Type species: *Azotomonas insolita* Sta

NOTES: Only two species are describ They are rods 0.6 to 1.2 μ by 0.6 to 5.0

Good growth occurs on meat infusi agar. Colonies are whitish, entire, and m be iridescent (*A. insolita*) or produce diffusible yellow or violet pigment (*A. fl rescens*).

The only other common characteris is the hydrolysis of starch. *A. insol* shares with *Aeromonas* spp. the character tic of gas production from glucose and la tose which is rarely found in polar flag lates.

Acid is produced from alcohol by *fluorescens* but the extent is not stated (*Acetobacter*).

e organisms are not known to utilize ol, or oxidize thiosulfates autotroph- , or hydrolyze agar or cellulose.

und in soil.

momonas (Zy.mo.mo′nas) Kluyver van Niel, 1936. *M p. 199.* (Zentr. eriol. Parasitenk., Abt. II, *94*, 1936,

FFERENTIATING CHARACTERS: Free-liv- unicellular, colorless, straight, non- ng, rod-shaped organisms up to 2 μ idth; motile with polar flagella; hetero- ic; grow poorly on organic media de- of yeast extract and carbohydrates; *ent glucose with the production of up to er cent ethyl alcohol,* some lactic acid, carbon dioxide; anaerobic to micro- philic; Gram-negative.

ey are not known to fix nitrogen or sit iron oxides in or on the cells.

pe species: *Zymomonas mobilis* (Lind- Kluyver and van Viel.

TES: Only two species are described. y are rather thick rods, 1 to 2 μ by 2 μ, occurring in pairs or small clumps. olonies on glucose yeast extract malt are approximately 1 mm. in diameter, lar, convex, and creamy to white by cted light and brownish by transmitted .

elatin is not liquefied.

e optimal temperature is 30° C.

und in fermenting plant juices.

otaminobacter (Pro.ta.mi.no. ter) den Dooren de Jong, 1926. *M p.* (Bijdrage tot de Kennis van het Min- satieproces. Thesis, Rotterdam, 1926,)

FFERENTIATING CHARACTERS: Free-liv- unicellular, nonsporing, nonphotosyn- ic, rod-shaped organisms less than 2 μ e; motile with polar flagella or non- ile; heterotrophic; capable of using al- mines as the sole source of carbon and ogen. *Aerobic*; catalase-positive; Gram- tive.

hey *do not or are not known to:* deposit oxides in or on the cells; hydrolyze tin, coagulated serum, cellulose, agar, chitin, or alginates; utilize phenol; oxidize ethyl alcohol* quantitatively to acetic acid; oxidize thiosulfates autotrophically; or fix gaseous nitrogen.

Type species: *Protaminobacter alboflavus* den Dooren de Jong, 1926.

NOTES: Only two species are described. Both grow on meat infusion agar and alkylamine agar producing opaque colonies which may be white, yellow, or red. The pigment produced is not prodigiosin.

Ability to use alkylamines may be lost in cultures maintained for long periods on meat infusion media.

The optimal temperature is 30° C.

Found in soil and water.

Alginomonas (Al.gi.no.mo′nas) Thjøtta and Kåss, 1945. *M p. 202.* (Avhandl. Norske Videnskaps-Akad., Oslo. I. Mat.-Naturv. Kl., No. 5, 1945, 17.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonsporing, colorless (or black), straight rod-shaped organisms less than 2 μ wide; motile with polar flagella; heterotrophic; capable of hydrolyzing and metabolizing alginates. *Aerobic.* Gram-negative.

They *do not or are not known to:* hydrolyze cellulose or chitin,† oxidize ethyl alcohol in appreciable amounts to acetic acid, produce ethyl alcohol in appreciable amounts from glucose, fix gaseous nitrogen, utilize phenol, or oxidize thiosulfates autotrophically.

Type species: *Alginomonas nonfermentans* Thjøtta and Kåss, 1945.

NOTES: Five species are described, three of which are of marine origin and require sea water media for growth.

They are rods 0.5 to 1.0 μ by 1 to 2 μ. Two species are capsulated.

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* Although no appreciable amount of acetic acid is produced from ethyl alcohol (?), the latter is used as a carbon source. A wide range of fatty and hydroxy acids can be metabolized when ammonia is used as a nitrogen source.

† Chitin hydrolysis is not mentioned in descriptions. One local isolate was found to hydrolyze both alginates and chitin.

Gelatin is liquefied by 4/5 species.

Agar is liquefied by 2/5 species.

Carbohydrates are stated to be "not utilized," although the fact is not mentioned in descriptions of 4 out of 5 species, and *A. terrestralginica* is reported to produce acidity in milk.

Optimal temperature 20°, 30°, or 37° C.

Found in soil and fresh sea water.

Mycoplana (My.co.pla′ na) Gray and Thornton, 1928. *M p. 204.* (Zentr. Bakteriol. Parsitenk., Abt. II, *73*, 1928, 82.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonsporing, colorless or black, straight or curved, rod-shaped organisms which may exhibit rudimentary branching; less than 2 μ wide; motile with polar flagella; heterotrophic; *capable of using phenol as a sole source of carbon* in the presence of a suitable nitrogen source; reduce nitrates, liberating nitrogen; no acid is produced from glucose or other carbohydrates; *Aerobic*, facultative; Gram-negative.

They *do not or are not known to:* hydrolyze cellulose, oxidize thiosulfate autotrophically, produce acetic acid quantitatively from ethyl alcohol, produce alcohol from glucose, fix gaseous nitrogen, or deposit iron oxides in or on the cells.

Type species: *Mycoplana dimorpha* Gray and Thornton.

NOTES: There are only two species, differentiated on gelatin liquefaction. They are rods, 0.5 to 1.0 μ by 1.5 to 4.5 μ, showing a tendency towards rudimentary branching. This is possibly a pleomorphic state.

Good growth of circular, smooth, glistening, bright colored colonies is produced on meat extract agar.

The optimal temperature is below 30° C.

Found in soil.

Zoogloea (Zo.o.gloe′ a) Cohn, 1854. *M p. 206.* (Nov. Act. Acad. Caes. leop.-carol. Nat. Cur., *24*, 1854, 123.)

DIFFERENTIATING CHARACTERS: Unicellular, colorless, nonsporing, straight, rod-shaped organisms, less than 2 μ wide, which characteristically develop in l
numbers in a firm gelatinous matrix; Gr
negative; free cells motile with polar
gella or nonmotile; *Aerobic*; heterotrop

They *do not or are not known to:* dep
iron in or on the cells;* hydrolyze cellul
agar or alginates; utilize alkylamines
phenol; oxidize ethyl alcohol quantitati
to acetic acid; produce alcohol from
cose; fix gaseous nitrogen; or produce a
from carbohydrates. They are not h
philic.

Type species: *Zoogloea ramigera* It
sohn.

NOTES: Two species are described. T
are rods 0.8 to 1.0 μ by 1 to 4 μ. In th
natural environment they usually deve
as a lobate gelatinous growth, that of
ramigera being broadly lobate whereas t
of *Z. filipendula* is made up of long narr
fingers. On agar media, small hemisphe
cal, cartilaginous colonies are produc
The natural formation is difficult to
produce even when growth occurs
der water and is almost certainly due to
jury to the mass during development.

Found in water and sewage and activat
sludge.

Halobacterium (Ha.lo.bac.te′ ri.u
Elazari-Volcani, 1940. *M p. 207.* (Stud
on the Microflora of the Dead Sea. Thes
Hebrew Univ., Jerusalem, 1940.)

DIFFERENTIATING CHARACTERS: Free-l
ing, unicellular, nonsporing, nonphotosy
thetic, spherical to rod-shaped organism
motile with polar flagella or nonmoti
heterotrophic; obligate halophiles which
quired 12 to 30 per cent salt for growt
Gram negative; *aerobic.*

They *do not or are not known to:* hydrol
cellulose or agar, utilize phenol, oxid
thiosulfates autotrophically, oxidize et
alcohol quantitatively to acetic acid, p
duce appreciable amounts of alcohol fr
glucose, or deposit iron in or on the ce

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* Although pure cultures do not depo
iron from iron-bearing waters, the natu
growths are frequently iron encrusted. T
is attributed to the activities of other org
isms.

ype species: *Halobacterium salinarium* ari-Volcani.

TES: Five species are described. They ormally rods, 0.5 to 1.0 μ by 1 to 8 μ; emely pleomorphic, with the shape ing with the medium and the specific content. Spherical cells are common icularly with variation in magnesium ent. Gas vacuoles occur in *Halobac-* *m halobium.*

he spherical cells are not motile.

rowth occurs on peptone media only n the salt content exceeds 16 per cent. er growth occurs with some species 24 per cent salt. Colonies range in di- ter from 1 to 6 mm. and are orange, w, red, or purple. The pigments of e species have been tested and yield a color with sulfuric acid, suggesting tenoids.

elatin is liquefied by 2/5.

dole is not produced.

$_2$S production is recorded as positive 2/5 species.

cid is produced from glucose by *H. ismortui.*

actose is not fermented.

tarch is not hydrolyzed (4/5).

itrates are reduced by three species, of which produce gas.

he optimal temperature is 30° C. (for species) or 37° (for four).

he organisms are responsible for the luction of discoloration in hides, fish, salt. There natural habitat is sea water brines.

iderocapsa (Si.de.ro.cap′sa) Mo- n, 1909. *M p. 218.* (Ann. Jard. bot. tenz. 2 Sér., Supp., 3, 1909, 29.)

IFFERENTIATING CHARACTERS: Unicel- r, colorless, nonsporing, spherical* or- isms 1 to 2 μ wide; 2 to 60 or more cells ur at random in a primary capsule 10 0 μ wide; secondary capsules unite to n a mucilaginous colony; iron or manganese compounds are stored in the secondary capsule. Heterotrophic.†

Type species: *Siderocapsa treubii* Molisch.

NOTES: Six species differentiated on morphological and ecological characters are described. The type species is found only in alkaline waters—a condition under which the true iron oxidizer like *Ferrobacillus* does not flourish.

Found mainly on the surfaces of growing plants in iron-bearing waters.

GENERAL NOTES: This genus and a number of the following genera are at best of doubtful significance. When surface scums from natural waters and decaying plant material are examined, isolated bacterial cells or groups of cells heavily encrusted with iron are frequently encountered. Iron encrustation is common also to numerous algae and some loricate and testate protozoa. Inorganic salts of ferrous iron are insoluble above pH 5.5, and inorganic salts of ferric iron are insoluble above a pH of approximately 2.3. In the neutral and alkaline environment of most natural waters, the only iron salts which occur in solution are organic. A typical salt is ferrous ammonium citrate.

Halliday and Grau (Nature, *179*, 1957, 733) have shown that when ferrous sulfate and citric acid are mixed, ferrous citrate is formed and the citrate catalyzes the oxidation of the ferrous iron to the ferric state while undergoing decarboxylation. Thus ferrous ammonium citrate, commonly used as a source of reduced iron, remains so for only a short period (less than 1 hour) so that in normal circumstances it is completely oxidized to the ferric state before the medium is inoculated. Further *oxidation* cannot occur. It would appear that in neutral and alkaline waters the iron-depositing organisms release the iron by metabolizing the organic substance with which it is chelated. The free ferric ion is either converted to the hydroxide or chelates with a

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Siderocapsa major is described as a rod- ped cell. It would be more logical to place the genus *Sideromonas.*

† None has been isolated. The heterotrophic nature is only an assumption.

component of the cell. Baas Becking suggests that pectin is one such substance which he claims occurs in the sheath of *Sphaerotilus*.

Almost any cell which metabolizes the citrate will deposit iron around itself in a ferric ammonium citrate medium. Hence the validity of the genus *Siderocapsa* of the following iron-depositing genera is in doubt.

Siderosphaera (Si.de.ro.sphae′ ra) Beger, 1944. *M p. 220.* (Ber. deut. botan. Ges., *62*, (1944) 1950, 7.)

DIFFERENTIATING CHARACTERS: Unicellular, colorless, nonsporing, spherical cells, occurring in pairs with one or more pairs embedded in a common capsule. Oxides of iron are deposited on the surface of the capsules.

Type species: *Siderosphaera conglomerata* Beger.

NOTES: Only one species has been described. It has not been isolated. Found on the surface of mud in bogs of the Austrian Alps.

See also general notes to *Siderocapsa*, p. 67.

Sideronema (Si.de.ro.ne′ ma) Beger, 1941. *M p. 220.* (Zentr. Bakteriol. Parasitenk., Abt. II, *103*, 1941, 321.)

DIFFERENTIATING CHARACTERS: Unicellular, colorless, nonsporing, ovoid to rod-shaped cells, 4.8 to 5.0 μ by 6.5 μ, forming short chains embedded in a thin mucilaginous capsule. Iron or manganese is deposited in the surface membrane of the cells rather than in the capsules.

Type species: *Sideronema globuliferum.*

NOTES: Only one species is described. It has not been isolated. The organism is nonmotile and was found on a glass slide submerged in spring water near Magdeburg, Germany.

See also general notes to *Siderocapsa*, p. 67.

Ferribacterium (Fer.ri.bac.te′ ri.um) Brussoff, 1916. *M p. 221.* (Zentr. Bakteriol. Parasitenk., Abt. II, *45*, 1916, 547.)

DIFFERENTIATING CHARACTERS: Fr living, unicellular, colorless, rod-sha organisms less than 2 μ wide arranged pairs or in chains; capsulated; oxides of i or manganese are deposited in the caps but a torus is not formed. Heterotrophi

Type species: *Ferribacterium du* Brussoff.

NOTES: Two species have been describ One species has been cultivated on p infusion agar, in iron ammonium citr broth, and in iron peptone broth, but o morphological and cultural data have b given.

Found forming a pellicle on iron-bear waters.

See also general notes to *Siderocapsa* 67.

Sideromonas (Si.de.ro.mo′ nas) Ch lodny, 1922. *M p. 222.* (Ber. deut. bota Ges., *40*, 1922, 326.)

DIFFERENTIATING CHARACTERS: Unicel lar, colorless, rod-shaped organisms l than 2 μ wide; capsulated and arranged random, in pairs or in chains in zooglo masses; the primary capsules are impre nated with iron or manganese oxid Heterotrophic.*

Type species: *Sideromonas conferva* Cholodny.

NOTES: Four species are described in t *Manual.* In two the cells are distinc listed as occurring in pairs or in chains a in the other two, as occurring at rando The genus *Ferribacterium* is differentiat from *Sideromonas* in the *Manual* ke (p. 217) on the formation of chains a pairs. Some rearrangement is obviou needed unless there exists a more suitab differentiating character, which is not o vious from the *Manual* descriptions unl zoogloeae are not formed by the genus *F ribacterium.*

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* It is suggested that some species a autotrophs or facultative autotrophs. Sin no information on the pH of the waters given, it is difficult to comment, but shou they be acid-tolerant and autotrophic t relationship to *Ferrobacillus* needs examin tion.

e also general notes to *Siderocapsa* p.

umanniella (Nau.man.ni.el′ la)
r, 1934. *M p. 223.* (Die Eisenorganis-
. Pflanzenforschung, Jena, Heft 16,
, 19.)

FFERENTIATING CHARACTERS: Unicel-
, colorless, nonsporing, rod-shaped
nisms which may exceed 2 μ in width,
nd which iron is deposited in the form
solid ring or *torus* which gives the cell
ppearance of a link in a chain; hetero-
hic.*

ype species: *Naumanniella neustonica,*
ff, 1934.

TES: Five species are described which
differentiated on morphology, size, and
ngement of the cells.

ound in surface films and bottom muds
on-bearing waters.

e also general notes to *Siderocapsa* p.

chrobium (O.chro′ bi.um) Perfiliev,
1 *M p. 225.* (In Wislouch, Bull. Inst.
lrobiol., Russia, 1921.)

IFFERENTIATING CHARACTERS: Unicellu-
colorless, ovoid to rod-shaped, non-
ring organisms which may exceed 2 μ in
th; partially surrounded by a solid ring
rus of iron giving the cell the appear-
of an open link in a chain; motile with
flagella of unequal length which emerge
the "open" end of the torus. Hetero-
hic.†

ype species: *Ochrobium tectum* Perfiliev.

OTES: Only one species is described. It
not been isolated. The cells measure
to 3.0 μ by 1.5 to 5.0 μ. It resembles the
, *Pteromonas* and because of the unusual
e of flagellation may, in fact, be an algal
.

See also general notes to *Siderocapsa*, p. 67.

Siderococcus (Si.de.ro.coc′ cus) Dorff, 1934. *M p. 225.* (Die Eisenorganismen, Pflanzenforschung, Jena, Heft 16, 1934, 9.)

DIFFERENTIATING CHARACTERS: Free-living, colorless, unicellular, spherical organisms less than 2 μ in diameter; not capsulated; oxides of iron or manganese deposited on the cells.

Type species: *Siderococcus limoniticus* Dorff.

NOTES: Two species are described. The cells are small, 0.2 to 1.0 μ in diameter, and occur singly or in chains. Neither species has been isolated in pure culture. In Dorff's original description of *S. limoniticus*, objects immersed in water overlayering slime from bog ore deposits became coated with a deposit of iron in a zone about equidistant from the slime and the surface of the water in the container.

S. communis is frankly described as heterotrophic; it deposits iron through the metabolism of citrate from ferric ammonium citrate. It seems likely that *S. limoniticus* is also heterotrophic.

See also general notes to *Siderocapsa*, p. 67.

Siderobacter (Si.de.ro.bac′ ter) Naumann. *M p. 226.* (Kgl. Svenska Vetenskapsakad. Handl., *62*, No. 4, 1922, 55.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, rod-shaped organisms which may exceed 2 μ in width; not capsulated; deposit oxides of iron directly on the cells; heterotrophic.

Type species; *Siderobacter linearis* Naumann.

NOTES: Five species are described, none of which has been isolated. Only one species, *S. latus*, has cells exceeding 1.0 μ in width. Differentiation is based solely on morphology and arrangement.

Found as surface growths on *Zoogloea filipendula* and on other submerged objects.

See also general notes to *Siderocapsa*, p. 67.

No indication is given of the auto- or
erotrophic nature of these organisms,
none has been isolated. Forms which
r a striking resemblance to illustrations
Naumanniella have been observed by
u in this laboratory in cultures of *Sphae-*
lus grown in a peptone medium contain-
an iron nail.

Heterotrophism is assumed.

Ferrobacillus (Fer.ro.ba.cil′lus) Leathen and Braley, 1954. *M p. 227.* (Bacteriol. Proc., 54th General Meeting, Soc. Am. Bacteriologists, 1954, 44.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, straight, rod-shaped organisms less than 2 μ wide; motile with polar flagella; obligate autotrophs which will not grow on meat infusion agar or other complex organic media; CO_2 used as a source of carbon and energy obtained from the oxidation of ferrous to ferric iron. Thiosulfate is not oxidised. *Aerobic.*

Type species: *Ferrobacillus ferrooxidans,* Leathen and Braley.

NOTES: A single species is described. The organism is a rod 0.6 to 1.0 μ by 1.0 to 1.6 μ; Gram-negative. It has been isolated in pure culture. It is a strict autotroph which oxidizes ferrous iron at a pH of 2.2 to 4.6 in which range ferrous but not ferric iron is soluble. The ferric iron produced by the oxidation is precipitated on the cells and in the medium.

It differs from *Thiobacillus ferrooxidans* in its inability to oxidize thiosulfate. The two organisms represent the only true "iron oxidizers" at present known.

See also general notes to *Siderocapsa,* p. 67.

Vibrio (Vib′ri.o) Müller, 1773. *M p. 229.* (Vermium terrestrium et fluviatilum, *1*, 1773, 39.)

DIFFERENTIATING CHARACTERS: Free-living unicellular, nonsporing, nonphotosynthetic, rod-shaped organisms with a curved (not spiral) axis; motile with polar flagella; Gram-negative; heterotrophic.

They *do not or are not known to:* deposit sulfur internally; hydrolyze cellulose (except *V. agarliquefaciens* and *V. fuscus*); oxidize thiosulfates autotrophically; utilize phenol (except *V. cyclosites*); produce water-soluble (except *V. fuscus*) or other pigments (except *V. extorquens* and *V. hyphalus*); oxidize ethyl alcohol quantitatively to acetic acid; produce alcohol from glucose; fix gaseous nitrogen; reduce sulfates to sulfides or deposit iron oxides the cells.

Type species: *Vibrio comma* (Schroet Winslow *et al.*

NOTES: Thirty-four species have b described. They are differentiated on o gen tolerance, acid production from car hydrates, bioluminescence (four species liquefaction of gelatin, indole producti reduction of nitrates, coagulation of mi pigment production (three species), s tolerance (four), hydrolysis of cellul (two) and agar (five), metabolism of nap thalene (one) and oxalates (two), path genicity to animals, and temperature growth.

Most of the organisms grow well meat infusion agar and many tolerate unusually high pH. The type species w grow at a pH of 9.0.

Four of the five anaerobic species a pathogenic to man and animals. They cau foul-smelling infections of the alimenta and urogenital tracts and other deep seat infections. They require the addition blood or ascitic fluid.

Six of the aerobic species cause diseas in man, animals, and insects. *V. comma* the etiologic agent of Asiatic cholera.

Desulfovibrio (De.sul.fo.vib′ri. Kluyver and van Niel, 1936. *M p. 24* (Zentr. Bakertiol. Parasitenk., Abt. *94*, 1936, 369.

DIFFERENTIATING CHARACTERISTI Free-living, unicellular, colorless, nonspo ing,† rod-shaped organisms with curv axes (not spiral); less than 2 μ wide; mot with a single polar flagellum; Gram-neg tive; *anaerobic;* heterotrophic; reduce su fates, sulfites, sulfur, thiosulfates, a hyposulfites to H_2S with the use of a wi range of organic substances and possib

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* Spencer (J. Gen. Microbiol., *13*, 195 111) draws attention to curved forms in t genus *Photobacterium.*

† Spore formation in *Desulfovibrio desu furicans* has been reported but the stat ment has since been withdrawn.

ogen as the hydrogen donor; do not
sit iron oxides.
pe species: *Desulfovibrio desulfuricans*
erinck, 1895) Kluyver and van Niel,
.

TES: Three species are described. They
lifferentiated on salt tolerance and the
re of the organic hydrogen donors.
e organisms are strict anaerobes which
apable of reducing sulfates with liber-
of hydrogen sulfide. In media con-
ing iron salts this results in the pro-
ion of black iron sulfide.
rimary isolation has been most success-
n a peptone-yeast extract-lactate me-
deoxygenated with a little sodium
de. Claims have been made that the
nisms will grow under strictly auto-
hic conditions with oxidation of H_2 for
gy.
he three species recorded do not liquefy
tin or reduce nitrates. No record is
le of acid production but all species
ze glucose, ethanol, propanol, butanol,
erol, lactate, malate, and several amino
s as hydrogen donors when reducing
inorganic sulfur compounds.
lthough enrichment cultures are easily
ntained subculture has proved very
cult.

ethanobacterium (Me.tha.no.-
te′ ri.um) Kluyver and van Niel,
. *M p. 250.* (Zentr. Bakteriol. Para-
k., Abt. II, *94*, 1936, 399.)

TRODUCTORY NOTE: Two organisms
ear in the *Manual* with the generic
gnation *Methanobacterium*. One, *M.*
ngenii Barker, has been listed as the
species of the genus *Methanobacterium*.
other, *M. omelianski*, has been listed
an appendix to the genus *Clostridium*
693). Both organisms occur in soil,
age, and fresh water *or* marine muds.
he characteristics of both are given.
also P. H. Smith and R. E. Hungate,
acteriol., *75*, 1958, 713.)
ethanobacterium soehngenii:

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, rod-shaped organisms less than 2 μ wide with a straight or slightly curved axis; occur in chains in liquid media; nonmotile; Gram-negative; *anaerobic*; heterotrophic; acetate and *n*-butyrate fermented with the production of CO_2 and methane; ethyl alcohol, *n*-butyl alcohol, and glucose not fermented.

Type species: *Methanobacterium soehngenii* Barker, 1936.

2. Methanobacterium omelianski:

DIFFERENTIATING CHARACTERS: Free-living unicellular, colorless, unbranched, rod-shaped organisms less than 2 μ wide; *endospores produced*; *anaerobic*; heterotrophic; oxidize primary alcohols, including ethyl alcohol and *n*-butyl alcohol, to the corresponding fatty acids and secondary alcohols to ketones while reducing CO_2 quantitatively to methane; sulfate is not reduced and sulfur is not deposited internally.

Cellvibrio (Cell.vib′ ri.o) Winogradsky, 1929. *M p. 250.* (Ann. inst. Pasteur, *43*, 1929, 577.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, nonsporing, rod-shaped organisms with a curved (not spiral) axis; less than 2 μ wide; Gram-negative; *aerobic*; grow moderately well on mineral salts agar containing starch; produce a yellow, ochre, or colorless growth on cellulose strips on a mineral salts agar and oxidize* the cellulose to oxycellulose; do not produce a clearing around colonies on precipitated cellulose agar nor cause disintegration of filter paper in 0.5 per cent peptone water.

They *do not or are not known to*: oxidize thiosulfates autotrophically, utilize phenol, oxidize ethyl alcohol quantitatively to acetic acid, produce alcohol in quantities from glucose, fix gaseous nitrogen, or deposit iron.

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* See the discussion of the validity of this statement in Methods, p. 157.

Type species: *Cellvibrio ochraceus* Winogradsky.

NOTES: Four species are described. They are differentiated on their ability to grow on glucose and starch agars and on pigmentation. Optimal temperature, 20° C.

Found in decaying vegetable matter and soils.

Cellfalcicula (Cell.fal.ci′ cu.la) Winogradsky, 1929. *M p. 252.* (Ann. inst. Pasteur, *43*, 1929, 616.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, nonsporing, sickle-shaped or curved rods less than 2 μ wide; motile with a single polar flagellum; Gram-negative; heterotrophic; *aerobic*; do not grow on a mineral salts medium containing starch; grow on cellulose strips layered on a minerals salts agar, oxidizing the cellulose to oxycellulose* and producing a green, cream, or brownish growth; do not cause disintegration of cellulose strips in peptone water.

They *do not or are not known to:* utilize phenol, oxidize thiosulfate autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce ethyl alcohol from glucose, fix gaseous nitrogen, produce water-soluble pigments, or deposit iron.

Type species: *Cellfalcicula viridis* Winogradsky.

NOTES: Three species are described.

Microcyclus (Mic.ro.cyc′ lus) Ørskov, 1928. *M p. 253.* (Zentr. Bakteriol. Parasitenk., Abt. I, Orig., *107*, 1928, 180.)

DIFFERENTIATING CHARACTERS: Free-living unicellular, colorless, curved, rod-shaped organisms about 1 μ long. During growth they produce closed rings, 2.0 to 3.0 μ in diameter, which later change to two horseshoe-shaped halves fastened together without any evidence of divisional lines. These divide into separate rods which reproduce the cycle; capsulated; nonmotile; Gram-negative; *aerobic*; heterotrophic—grow well in 0.5 per cent peptone water.

They *do not or are not known to:* utilize phenol, oxidize thiosulfate autotrophica
oxidize ethyl alcohol quantitatively
acetic acid, produce alcohol from gluco
fix gaseous nitrogen or deposit iron.

Type species: *Microcyclus aquati*
Ørskov.

NOTES: Only one species has been
scribed.

It does not produce acid from hexo
but produces slight acid from pentoses.

Gelatin is not liquefied and indole is
formed.

Temperature range, 5° to 30° C.

Found in lake water and soil.

Spirillum (Spi.ril′ lum) Ehrenberg. 18
M p. 253. (Physik. Abhandl. Akad. Wis
Berlin, 1832, 38.)

DIFFERENTIATING CHARACTERS: Fr
living, unicellular, nonsporing, nonpho
synthetic, rod-shaped organisms with
spiral axis and a relatively rigid cell wa
less than 2 μ wide; motile with polar fla
ella; Gram-negative; heterotrophic; do n
deposit sulfur internally.

Type species: *Spirillum undula*(Mülle
Ehrenberg.

NOTES: Nine species have been describ
in the *Manual.* They are differentiated
morphological and cultural characteristi
The spirilla are common in putrid a
stagnant waters. One species, *S. minus,*
considered to be a cause of rat-bite feve

The genus *Spirillum* has recently be
redescribed in a monograph by Willia
and Rittenberg (Intern. Bull. of Bacteri
Nomen and Taxon, *7*, 1957, 49). Th
note that species of *Spirillum* are capa
of changing from the characteristic spi
form to a coccoid body in aging cultur
These coccoid bodies germinate in a fre
medium to give the normal spiral form
The cultures are very sensitive to Na
This claim has been verified in this labor
tory. Four cultures supplied by Willia
have grown well on a Difco peptone-yea
extract agar without the usual 0.5 per ce
salt.

The suggested relationship betwe
Spirillum and the myxobacteria seems

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* See comment on the validity of this statement in Methods, p. 157.

. The formation of flagella by the
es of *Spirillum* sets them aside from
onflagellated creeping myxobacteria.

raspirillum (Pa.ra.spi.ril′ lum) Do-
1912. *M p. 257.* (Arch. Protistenk.,
912, 97.)

FFERENTIATING CHARACTERS: Free-
g, unicellular, colorless, nonsporing
nisms with a spiral axis; rather sharply
lated; cells 1.5 to 2.0 μ wide at the
er and tapering towards both ends. In
enter of the cell is an oval to rounded
almost as wide as the cell and clearly
le without staining. It stains deeply
neutral red in killed cells and with
lenhain's iron-hematoxylin or Giemsa
in fixed cells and is considered by
ell to be a nucleus; motile with polar
lla; when attached to an object by one
the cells are capable of contraction to
ore angulated spiral; do not deposit
ur internally.

ype species: *Paraspirillum vejdovskii*
ell.

OTES: A single species is described. Cells
8 to 15 μ long. The "well defined nu-
s" described by Dobell is a most un-
al character for a bacterial cell. Similar
have been found in local waters in
h the central "nucleus" looked like a
green chloroplast. It may be an algal
(author's note). It has not been iso-
l.

elenomonas (Se.le.no.mo′ nas) von
wazek, 1913. *M p. 258.* (Zentr. Bak-
l. Parasitenk., Abt. I, Orig., *70*, 1913,

FFERENTIATING CHARACTERS: Free-
g, unicellular, colorless, curved, rod-
ed organisms which may exceed 2 μ in
th; motile by means of a bunch of flag-
inserted in the concave side of the cell;
sporing; Gram-negative; heterotrophic;
erobic; do not deposit sulfur or calcium
onate internally or iron externally.

ype species: *Selenomonas palpitans*
ons.

OTES: The organisms occur in the ru-
, cecum, and buccal cavities. Only one species has been isolated (from the buccal cavity of man). It requires enriched media and produces on blood agar a smooth, convex, grayish yellow colony less than 0.5 mm. in diameter. It produces acid from glucose and sucrose and acidifies and coagulates milk. It does not produce H_2S or indole or reduce nitrates.

Several authors have claimed that *Selenomonas* belongs to the *Protozoa*. Electron micrographs of flagella should resolve this question.

Myconostoc (My.co.nos′ toc) Cohn, 1875. *M p. 260.* (Beitr. Biol. Pflanz., *1*, Heft 3, 1875, 183.)

DIFFERENTIATING CHARACTERS: Unicellular, colorless, nonsporing, curved rods less than 2 μ wide; arranged in chains wound into a ball inside a nearly spherical capsule; polar flagellated swarm cells are produced. Gram-negative (?); heterotrophic (?).

They *do not or are not known to:* utilize phenols, oxidize thiosulfates autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce alcohol from glucose, fix gaseous nitrogen, or deposit iron about the cells or capsules.

Type species: *Myconostoc gregarium* Cohn, 1875.

NOTES: Only one species is described. It has been found in the surface scum of sulfur water in a jar of decomposing algae and in peat bogs. Not isolated.

Pasteurella (Pas.teu.rel′ la) Trevisan 1887. *M p. 395.* (Rend. reale ist. lombardo sci., 1887, 94.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, ovoid to straight, rod-shaped organisms less than 2 μ wide; nonmotile or motile with flagella; nonsporing; Gram-negative; *aerobic*; heterotrophic; no gas from carbohydrates. Parasitic on man, animals, and birds.

They *do not or are not known to:* hydrolyze cellulose or agar, liquefy Loeffler's inspissated serum, oxidize thiosulfate autotrophically, utilize phenol, oxidize ethyl alcohol quantitatively to acetic acid, produce ethyl alcohol from glucose, fix gaseous nitrogen, or deposit iron.

Type species: *Pasteurella multocida* (Lehmann and Neumann) Rosenbusch and Merchant.

NOTES: Organisms 0.1 to 0.7 μ by 0.3 to 1.7 μ, generally ellipsoidal, often showing a bipolar stain. Nonmotile except for *P. pseudotuberculosis* which is motile below 30° C. and has peritrichous flagella.

All species excepting *P. tularensis* and *P. novicida* grow on meat infusion agar; the colonies are small (1 mm.) in diameter, circular, translucent, and colorless to slightly yellowish in 24 hours at 37° C. Colonies of some species spread on further incubation and may have a draughtsman-like appearance.

P. tularenis grows on blood agar and *P. novicida* on yeast extract agar, both with colonies similar to the above.

Gelatin is slowly liquefied by 3/7 species.

Litmus milk remains unchanged or is slightly alkaline.

H_2S is produced by all species except *P. pfaffii.*

Indole is produced by only one species.

Acid but no gas is produced from glucose by all species except *P. anatipestifer*.

No acid is produced from lactose (except *P. haemolytica*).

Optimal temperature, 37° C. except for *P. pseudotuberculosis* (30° C).

PATHOGENICITY: The organisms are responsible for septicemic conditions characterized by the production of multiple small hemorrhages (petechiae) throughout the body.

P. multocida: a cause of chicken cholera and hemorrhagic septicemia in warm-blooded animals.

P. septicaemiae: fatal septicemia of young geese.

P. haemolytica: pneumonia of sheep and cattle.

P. pestis: bubonic and pneumonic plague in man.

P. pfaffii: epidemic septicemia in canaries.

P. pseudotuberculosis: causes pseudotub
culosis in a wide range of hosts.

P. tularensis: tularemia in man.

P. novicida: tularemia-like disease of
dents.

Bordetella (Bor.de.tel′ la) Moren
López, 1952. *M p. 402.* (Microbiol. espa
5, 1952, 177.)

DIFFERENTIATING CHARACTERS: Fr
living, unicellular, colorless, ovoid to ro
shaped organisms less than 2 μ in diamete
nonmotile or motile with flagella; Gra
negative; heterotrophic; no acid or gas pr
duced from glucose or lactose; litmus mi
made alkaline in 1 to 2 days; organism
produce whooping cough or related diseas
in children or bronchopneumonia in r
dents.

They *do not or are not known to:* hydroly
cellulose or agar, liquefy Loeffler's inspi
sated serum, utilize phenol, oxidize thi
sulfate autotrophically, oxidize ethyl alc
hol quantitatively to acetic acid, produ
alcohol from glucose, fix gaseous nitroge
or deposit iron.

Type species: *Bordetella pertussis* (Ho
land) Moreno-López.

NOTES: Minute, rod-shaped organism
0.2 to 0.3 μ by 1 μ. Gram-negative. Of th
three species only one, *Bordetella bronch*
septica, is motile. It has peritrichous flag
ella.

B. parapertussis and *B. bronchisepti*
will grow on first isolation on meat infusi
agar but the type species will not. It r
quires a different medium. The potat
blood-glycerol medium of Bordet ar
Gengou has proved most suitable. It ha
been replaced by a charcoal agar whic
suggests that the unsuitability of me
infusion agar may be due to a toxic com
ponent adsorbed on the charcoal rath
than a deficiency of some essential metab
lite.

Colonies are colorless, circular, conve
and entire and are about 1 mm. in diamet
in 24 hours.

tin is not hydrolyzed.

tmus milk turns alkaline very slowly.

dole is not produced.

trite is produced from nitrate by *B.* *chiseptica.*

acid or gas is produced from glucose ctose.

trate is used as a sole source of carbon urease is produced by *B. parapertussis* *B. bronchiseptica.*

talase-positive.

erobic; optimal temperature, 37° C.

. *pertussis* and *B. parapertussis* have isolated from cases of whooping cough related infections in man. *B. bronchi-* *ca* is isolated from dogs affected with mper.

rucella (Bru.cel′ la) Meyer and Shaw,). *M p. 404.* (J. Infectious Diseases, 1920, 173.)

IFFERENTIATING CHARACTERS: Free- g, unicellular, colorless, nonsporing, ight to ovoid, rod-shaped organisms than 2 μ in width; nonmotile; Gram- ative; heterotrophic. The organisms w well on liver extract or tryptose- sphate agar; no apparent acid or gas is duced from glucose or lactose in peptone er; litmus milk turns alkaline; Loeffler's issated serum is not liquefied.

hey *do not or are not known to:* hydrolyze lose or agar, utilize phenols, oxidize sulfate autotrophically, oxidize ethyl hol quantitatively to acetic acid, pro- alcohol from glucose, fix gaseous ogen, or deposit iron.

he organisms cause *brucellosis* in man animals and may cause abortion in nals.

ype species: *Brucella melitensis* ghes) Meyer and Shaw.

OTES: Three species are described. They differentiated on tolerance to the dyes onin and basic fuchsin, production of , and antigenic pattern.

rganisms are 0.2 to 0.3 μ by 0.5 to 1.0

rowth occurs on meat infusion agar but is improved by liver extract. An increased CO_2 concentration favors isolation of *B. abortus* but the optimal oxygen concentration is still 20 per cent.

Colonies are 1 mm. in diameter.

Gelatin is not liquefied.

Litmus milk becomes slowly alkaline.

Indole is not produced.

Nitrite is produced from nitrate.

No visible acid is produced from carbohydrates in peptone water, although glucose and other carbohydrates are reported to be oxidized. This is due to neutralization of the acid by ammonia liberated.

Aerobic. Optimal temperature, 37° C. Optimal pH, 7 to 7.2. No growth below pH 6.0.

Brucella abortus causes contagious abortion in cattle and undulant fever in man. It also affects swine. *Brucella melitensis* causes abortion in goats and undulant fever in man. It may affect swine. *Brucella suis* causes abortion in swine and undulant fever in man. It also affects chickens, horses, dogs, cows, and monkeys.

Haemophilus (Hae.mo′ phi.lus) Winslow *et al.*, 1917. *M p. 406.* (J. Bacteriol., *2*, 1917, 561.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, straight, rod-shaped organisms less than 2 μ in width; nonmotile; Gram-negative; heterotrophic; fastidious organisms which will not grow on meat infusion agar but require the addition of X- or V-factor (or both) or diphosphothiamine to support growth. No gas is produced from carbohydrates; acid may be produced from glucose but only rarely from lactose; not halophilic; parasitic.

They *do not or are not known to:* hydrolyze cellulose or agar, liquefy Loeffler's inspissated serum, utilize phenol, oxidize thiosulfate autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce alcohol from glucose, fix gaseous nitrogen, or deposit iron.

Type species: *Haemophilus influenzae* (Lehmann and Neumann) Winslow.

NOTES: Fifteen species are described with differentiation on pathogenicity and dependence on X- and V-factors. Although normally rod-shaped, the cells may become very pleomorphic, especially in pathological material.

The organisms do not grow on meat infusion agar except after adaptation. Individual species require the addition of hemin (X-factor) and phosphopyridine nucleotide (V-factor) for growth. X-factor is contained in blood and the V-factor, in yeast extract. The only exception is *Haemophilus piscium* which requires neither but does require diphosphothiamine or adenosine.

In practice a medium (known as Levinthal base) devoid of X- and V-factors is employed upon which these organisms will *not* grow. The addition of X- or V-factor or X- + V-factors determines their growth requirements. It should be remembered that when an organism will not grow on a meat infusion base it is useless to employ *peptone* as a base in media for biochemical tests. Careful examination must be made to check for growth before any assessment of activity is made.

Colonies on suitable media vary from tiny colorless colonies 0.5 mm. in diameter to iridescent colonies 2 to 3 mm. in diameter with different species. Colonies of *H. citreus* are lemon yellow.

Gelatin is not suitable for growth.

Indole is rarely produced (4/15).

Nitrite is produced from nitrate by all species.

Acid but no gas is produced from glucose by strains of all species, although strains frequently occur which will not produce acid from any sugars.

Acid produced from lactose by three species which also produce a slight acidity in litmus milk.

Blood is hemolyzed by only 3/15 species. Hemolysis may be either of an α (green) or β (colorless) type.

Optimal temperature is 37° C. fo but *H. piscium* which has an optimum tween 20° and 25° C. It causes ulcer trout.

PATHOGENICITY: Obligate parasites animals; not always pathogenic. Dise caused are listed below.

H. influenzae: purulent meningitis and junctivitis in man.

H. hemolyticus: subacute endocarditis.

H. aegyptius: acute and subacute conju tivitis.

H. ducreyi: Soft chancre.

H. citreus: genital vesicular eruptions cattle.

H. piscium: ulcers of trout.

H. haemoglobinophilus: respiratory tract man and preputial secretions of dogs.

H. parahemolyticus: pharyngitis.

H. parainfluenzae: nonpathogenic.

H. gallinarum: fowl coryza.

H. suis: associated with a virus in sw influenza.

H. putoriorum: respiratory tract of ferre

Actinobacillus (Ac.ti.no.ba.cil'l Brumpt, 1910. *M p. 414.* (Précis de Pa sitologie, 1st ed., Paris, 1910, 849.)

DIFFERENTIATING CHARACTERS: Fr living, unicellular, colorless, nonsporin rod-shaped organisms less than 2 μ width; nonmotile; Gram-negative; *aerob* heterotrophic; no gas produced from carb hydrates; production of acid from lact and glucose varies with the species. Anir parasites.

They *do not or are not known to:* hydroly cellulose or agar, *liquefy Loeffler's insp sated serum, require X- or V-factors growth,* utilize phenols, oxidize thiosulf autotrophically, oxidize ethyl alcohol qu titatively to acetic acid, produce alco from glucose, fix gaseous nitrogen, or d posit iron.

Type species: *Actinobacillus lignier* Brumpt.

NOTES: Often occur in clumps in tiss and cultures. Growth occurs on meat fusion agar without enrichment. The colo

varies with the species from small
o 1 mm.), smooth, entire, butyrous
es which may increase in size on
r incubation to small mucoid or carti-
ous colonies which adhere to the agar.
species produce a granular growth
ent to the walls of the tubes in liquid
.

nules obtained from lesions may have
crushed before primary isolation can
hieved.

atin is not liquefied.

mus milk may be turned slightly acid
a period of weeks.

ght acid production occurs in glucose
also in lactose with 4/5 species but
not be evident in 24 to 48 hours. No
produced.

timal temperature, 37° C.

THOGENICITY: Diseases caused in ani-
are listed below.

ignieresii: actinobacillosis in cattle;
s lesions mainly in soft tissues, par-
rly in lymph nodes and muscles of
ongue.

quuli: causes joint ill and purulent
ritis in foals.

ctinomycetocomitans: found associated
Actinomyces hominis in actinomycosis.

tinoides: chronic pneumonia in calves.

allei: glanders in horses.

lymmatobacterium (Ca.lym.ma.-
c.te′ri.um) Aragão and Vianna,
M p. 418. (Mem. inst. Oswaldo
, *5*, 1913, 221.)

FERENTIATING CHARACTERS: Free-
, unicellular, colorless, nonsporing,
ght, rod-shaped organisms less than
vide; nonmotile; Gram-negative; *aero-*
heterotrophic; isolated from cases of
uloma inguinale; growth does not occur
eat infusion agar on primary isolation;
be cultivated in the yolk sac of the
loping chick embryo or in the con-
ation water of a sloped medium pre-
l by adding 50 per cent unheated
yonic yolk to melted and cooled nu-
t agar; do not deposit iron.

Type species: *Calymmatobacterium granulomatis* Aragão and Vianna.

NOTES: There is only one species. Rod-shaped, pleomorphic, unicellular organisms 0.8 to 1.4 μ by 1.5 to 4.5 μ. Smaller cell types 0.5 to 0.7 μ by 1.4 to 1.5 μ occur. Single cells may be slightly curved. Encapsulated.

On primary isolation the organism will grow only on a medium containing unheated embryonic egg yolk but can later be adapted to Levinthal basal medium + X-factor (Rake and Oskay, J. Bacteriol., *55*, 1948, 667). Adapted forms produce a shiny translucent colony which grows to 1.5 mm. in diameter and turns gray, then brown. Colonies take 4 days to appear. Spasmodic growth occurs in beef heart broth without blood extract.

Good growth occurs in the yolk sac of the developing chick embryo.

Optimal temperature, 37° C. No further information on cultural and biochemical reactions is available.

The organisms are found in granulomatous lesions in man and are considered to be the cause of *granuloma inguinale.*

Moraxella (Mo.rax.el′la) Lwoff, 1939. *M p. 419.* (Ann. inst. Pasteur, *62*, 1939, 168.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, straight, rod-shaped organisms less than 2 μ wide; nonmotile; Gram-negative; *aerobic*; heterotrophic; *liquefies Loeffler's inspissated serum*; no acid or gas produced from glucose or lactose. Species cause ophthalmia (pink eye) of cattle, angular conjunctivitis in man, monkeys, and apes and possibly trachoma in man.

They *do not or are not known to:* hydrolyze cellulose or agar, utilize phenol, oxidize thiosulfate autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce alcohol from glucose, fix gaseous nitrogen, or deposit iron.

Type species: *Moraxella lacunata* (Eyre) Lwoff.

NOTES: Three species are described, differentiated in the *Manual* by their action on gelatin and litmus milk.

They are rod-shaped organisms, 0.4 to 0.5 μ by 1.5 to 2.0 μ.

Growth on meat infusion agar is poor. It is improved by the addition of serum or ascitic fluid.

Litmus milk does not support growth of 2/3 species. The third one turns it slightly alkaline.

Loeffler's inspissated serum is liquefied.

No acid is produced from carbohydrates, although it is stated that *M. lacunata* "attacks various carbohydrates and mannitol."

Optimal temperature, 37° C.

Noguchia (No.gu′ chi.a) Olitsky *et al.* 1934. *M p. 421.* (J. Exptl. Med., *60*, 1934, 382.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, straight, rod-shaped organisms less than 2 μ wide; motile with flagella; Gram-negative; heterotrophic; *aerobic*; no gas is produced from carbohydrates; acid production from glucose and lactose varies with the species; parasitic on warm-blooded animals, producing granular conjunctival folliculosis in rabbits.

They *do not or are not known to:* hydrolyze agar or cellulose; liquefy Loeffler's inspissated serum, utilize phenol, oxidize thiosulfate autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce alcohol from glucose, fix gaseous nitrogen, or deposit iron.

Type species: *Noguchia granulosis* (Noguchi) Olitsky *et al.*

NOTES: Three species are described. They are differentiated in the *Manual* on acid production from glucose, raffinose, maltose, and salicin.

Rod-shaped organisms, 0.2 to 0.3 μ by 0.5 to 1.2 μ. Motile with peritrichous flagella. (Flagellation is considered to be peritrichous, although in some instances only a single flagellum may be produced, located either laterally or at the pole).

Two out of three tested species grow meat infusion agar and the third on bl agar. Colonies are small grayish and tra lucent and may be mucoid with capsula organisms.

Litmus milk is not changed.

Gelatin is not liquefied.

Acid but no gas is produced from gluc and lactose by two species. *N. cuniculi* d not produce acid from sugars.

Nitrite is not produced from nitrate.

Indole is not produced.

Aerobic. Optimal temperature is betw 28° and 30° C. with all three species. M tility may not be present in cultures gro at higher temperatures.

Species are serologically homogeneous

PATHOGENICITY: All species are pat genic and cause the following diseases.

N. granulosis: considered to cause tracho in man.

N. simiae: conjunctival folliculosis in mo keys.

N. cuniculi: conjunctival folliculosis in ra bits.

Mima (Mi′ ma) de Bord, 1942. (Io State Coll. J. Sci., *16*, 1942, 471.)

DIFFERENTIATING CHARACTERS: Fr living, unicellular, colorless, nonspori organisms less than 2 μ wide which occ predominantly as diplococci on solid med on primary isolation but as a mixture, cocci and rods in liquids with the rod fo increasing in proportion as the culture ag encapsulated; nonmotile; Gram-negati *aerobic*; heterotrophic; grow well on me infusion agar; no acid or gas produced fr glucose or lactose.

They *do not or are not known to:* hydroly cellulose or agar, utilize phenol, oxid thiosulfate autotrophically, oxidize et alcohol quantitatively to acetic acid, pr duce ethyl alcohol from glucose, or depo iron.

Type species: *Mima polymorpha* de Bo

NOTES: This genus is not listed in t *Manual.*

Only one species has been described. T

rm of the organism is 0.5 to 0.7 μ by
3.0 μ; the cocci are 0.5 to 0.7 μ in
ter.

nies on meat extract agar are white,
ing, smooth, moist, and viscid.

thyl red-negative.

es-Proskauer-negative.

ate-negative.

ole-negative.

rites are not produced from nitrate.

alase-positive.

dase-negative.

nd in the normal vagina.

rellea (He.rel′ le.a) de Bord, 1942.
State Coll. J. Sci. *16*, 1942, 471.)

FERENTIATING CHARACTERS: Free-
, unicellular, colorless, nonsporing
isms less than 2 μ wide which occur
minantly as diplococci on solid media
imary isolation but as a mixture of
and rods in liquids with the rod form
sing in proportion as the culture ages;
sulated; nonmotile; Gram-negative;
c; heterotrophic; grow well on meat
ct agar; acid but no gas produced from
se; lactose not fermented.

ey *do not or are not known to:* hydrolyze
ose or agar, utilize phenol, oxidize
lfate autotrophically, oxidize ethyl
l quantitatively to acetic acid, pro-
ethyl alcohol from glucose, or deposit

e species: *Herellea vaginicola* de Bord.

ES: This genus is not listed in the
al. Only one species is described.
l forms are 0.5 to 0.7 μ by 1.0 to 3.0
ci are 0.5 to 0.7 μ in diameter. Col-
on meat extract agar are white,
ning, smooth, moist, and viscid.

d is produced from glucose, mannitol
lulcitol.

ethyl red-negative.

ges-Proskauer-negative.

trites not produced from nitrate.

rate is utilized.

talase-positive.

idase-negative.

und in the normal vagina and in cases
njunctivitis.

Colloides (Col.loi′ des or Col.lo.i′ des) de Bord, 1942. (Iowa State Coll. J. Sci., *16*, 1942, 471).

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing organisms, less than 2 μ wide, which occur predominantly as diplococci on solid media on primary isolation but as a mixture of cocci and rods in liquids with the rod form increasing in proportion as the culture ages; encapsulated; nonmotile; Gram-negative; *aerobic*; heterotrophic; grow well on meat extract agar; acid and gas produced from glucose and lactose; methyl red-positive; Voges-Proskauer-negative; not luminescent.

They *do not or are not known to:* hydrolyze cellulose or agar, utilize phenol, oxidize thiosulfate autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce ethyl alcohol from glucose, or deposit iron.

Type species: *Colloides anoxydana* de Bord.

NOTES: This genus is not listed in the *Manual*. Only one species is described. Rod forms are 0.5 to 0.7 μ by 1.0 to 3.0 μ; cocci are 0.5 to 0.7 μ in diameter. Colonies on meat extract agar are white, glistening, smooth, moist, and viscid.

Acid and gas produced from glucose, maltose, lactose, mannitol, and dulcitol. Sucrose, salicin, and dextrin are not fermented.

Indole-positive.

Nitrites produced from nitrates.

Catalase-positive.

Oxidase-negative.

Found in the normal vagina and in cases of conjunctivitis.

Sphaerotilus (Sphae.ro′ til.us) Kützing, 1833. *M p. 263.* (Linnaea, *8*, 1833, 385.)

DIFFERENTIATING CHARACTERS: Unicellular, colorless, straight, rod-shaped organisms which may exceed 2 μ in width. Occur in chains enclosed in a sheath of uniform width attached by means of a conspicuous holdfast; free cells motile with subpolar flagella.

Type species: *Sphaerotilus natans* Kützing.

NOTES: Three species have been described. They are rod-shaped cells, 2 by 3 to 6 μ and motile with subpolar flagella. Gram-negative. In the living cells of *S. natans* under dark ground illumination there appears to be only one flagellum of wide amplitude. In stained specimens this "flagellum" is found to separate into at least seven individual flagella.

Motile cells secrete a gum which fixes them to a surface, usually at the liquid-air junction. The cell is usually fixed with the long axis in a vertical position. Division is followed by further secretion of the capsular material. With further development the capsule resolves into a tubular sheath which is produced continuously as the chain of cells grows. A cell liberated from the tip or by rupture will start a new colony. Occasionally it adheres to the sheath of the original chain, and the new chain gives the appearance of a false branch. The cells and sheath are colorless in substrates free of organic iron compounds. In the presence of the latter, iron is deposited in the sheath and the organisms become indistinguishable from *Leptothrix*. Pringsheim (Phil. Trans. Roy. Soc. London, Ser. B, *233*, 1949, 605; and Biol. Revws., Cambridge Phil. Soc., *24*, 1949, 200) considers the genera identical. Beger (Zentr. Bakteriol. Parasitenk., Abt. II, *107*, 1953, 318) claims the sheaths are of different composition.

During the deposition of the iron the sheaths become thickened and hard and brittle. The cells within the basal portion of the sheath become progressively thinner and disappear (author's note).

Growth occurs readily on 0.1 per cent peptone in the form of filamentous colonies, the projecting edges of which show organisms within a clearly defined sheath. In the denser regions the sheath disappears. On richer media the organism may produce yeast-like colonies in which cells occur random in a mucilaginous mass.

Skerman, Dementjeva, and Carey Bacteriol., *73*, 1957, 504) demonstra that *Sphaerotilus natans*, when exposed H_2S deposits sulfur internally.

Leptothrix (Lep′to.thrix) Kützi 1843. *M p. 264.* (Phycologia Genera 1843, 198.)

DIFFERENTIATING CHARACTERS: Unicel lar, colorless, rod-shaped organisms l than 2 μ wide; occur in chains withi sheath which does not increase in wi *from the base to the tip*; divide by transve division only; extruded cells are of the sa dimensions as those in the sheath; depo oxides of iron or manganese within t sheath.

Type species: *Leptothrix ochracea* K zing.

NOTES: Twelve species, differentia purely on morphological grounds ha been described in the *Manual.*

The essential characteristic of spec within this genus is their ability to oxid iron, depositing the latter within t sheath. Apart from this character, they not differ from *Sphaerotilus* (see Notes *Sphaerotilus*).

AUTHOR'S NOTE: The form of motility a the general morphology of some spec placed in this genus are very vague. Th is a suggestion that some may show glid motility. It should be noted in this reg that when *Sphaerotilus natans* is grown 0.1 per cent peptone water containing fe ammonium citrate, the width of individ cells within the sheath varies very c siderably with the amount of iron deposi in the sheath. If a very long sheathed f ment is examined, the cells toward the where the sheath has recently been for and is still colorless, are fully developed a at least 2.0 μ wide. The cells becomes p gressively smaller in width towards older portion of the sheath and are reduc to thin rods only 0.5 μ wide in the reg where the sheath has become quite bro and brittle. Microscopic examination nts in this region has frequently re- the sudden disappearance of indi- cells within the chain followed by a harp movement of the rest of the along the rigid tube. This could quite be confused with the gliding move- characteristic of *Beggiatoa*. The nor- rminal cells, however, are actively when free and have clearly defined a.

othrix (Tox′ o.thrix) Molisch, 1925. *269.* (Sci. Repts. Tôhoku Imp. Univ. h Ser., 1925, 144.)

FERENTIATING CHARACTERS: Unicellu- olorless, rod-shaped organisms less μ in width, arranged in chains within th. With further development arched aped groups of sheathed organisms rmed; iron is deposited in the sheath. plication of the cells is by transverse on only, and sheaths do not increase lth from the base to the tip.

pe species: *Toxothrix trichogenes* sch) Beger.

ES: There are two described species. is a difference of opinion regarding ode of formation of the arched groups. ne species, *T. gelatinosa*, several hed chains of cells appear to arise approximately the same point and branching is common. The branches o arch outwards.

the other species, *T. trichogenes*, it is sted that splitting of the original , followed by continued development chain of cells, results in the forma- f the arched groups.

oploca (Pe.lo′ plo.ca) Lauterborn, *M p. 270.* (Allgem. botan. Z., *19*, 99.)

FERENTIATING CHARACTERS: Multicel- colorless, organisms, 2 μ or less in , in which each of the individual cells -shaped and contains a gas vacuole ome) which emits a reddish gleam in mitted light. Trichomes are arranged ndles; nonmotile; do not deposit iron ; do not oxidize sulfides to sulfur.

Type species: *Peloploca undulata* Lauterborn.

NOTES: Two species are recorded. No mention is made in descriptions of a sheath on either species, although this is a character employed for their differentiation in the Key to families of the Order *Chlamydobacteriales.*

Individual cells are 3 to 10 μ in length.

The descriptions in the *Manual* leave some doubt as to whether the organisms of this genus and that of *Pelonema* are chains of unicellular organisms or simply multicellular organisms like *Beggiatoa*. The formation of "trichomes" without sheaths, as described for *Peloploca*, may indicate a multicellular form, although it can well be argued that the same should apply to *Streptococcus*.

Pending clarification of this, the organisms of the genera *Pelonema* and *Peloploca* have been considered as multicellular.

Pelonema (Pe.lo.ne′ ma) Lauterborn, 1015. *M p. 271.* (Verhandl. naturhist.-medizin. Ver., Heidelberg, N. F., *13*, 1915, 408.)

DIFFERENTIATING CHARACTERS: Multicellular, colorless organisms, 2 μ or less in width, in which each of the individual cells is rod-shaped and contains a gas vacuole (aerosome) which emits a reddish gleam in transmitted light. Trichomes occur singly and are enclosed in a thin sheath;* do not deposit iron oxides; do not oxidize sulfides to sulfur.

Type species: *Pelonema tenue* Lauterborn.

NOTES: Four species are described which are differentiated on morphological characteristics. Individual cells range from 4 to 12 μ long. Trichomes may exceed 200 μ in length.

Found on the surface of waters containing decomposing algae and poorly aerated.

See also note to *Peloploca*.

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* The presence of a sheath, indicated in the description of the genus in the *Manual*, is not mentioned in any of the species descriptions.

Crenothrix (Cre′ no.thrix) Cohn, 1870. *M p. 272.* (Beitr. Biol. Pflanz., *1*, Heft *1*, 1870, 108.)

DIFFERENTIATING CHARACTERS: Colorless, unicellular* rod-shaped organisms arranged in chains within a sheath which increases in width from the base to the tip; cells in the basal portion of the sheath are longer than wide. Cells within the sheath vary in size and may exceed 2 μ in width. They divide transversely and longitudinally near the tip to yield a mass of coccoid elements which are nonmotile. When growing in iron or manganese-bearing waters, they deposit large quantities of iron or manganese within the sheath.

Type species: *Crenothrix polyspora* Cohn.

NOTES: Only one species is described. It has not been isolated.

Phragmidiothrix (Phrag.mi′ di.o. thrix) Engler, 1883. *M p. 273.* (Vierter Ber. d. Commission z. wissensch. Unters. d. deutsch. Meere in Kiel für 1877 bis 1881, I Abt., 1883, 187.)

DIFFERENTIATING CHARACTERS: Unicellular,† colorless, disc-shaped organisms arranged in chains within a sheath which increases in width from the base to the tip. Cells in the basal portion of the sheath are much wider than long and may exceed 2 μ in width. They divide longitudinally and transversely near the tip to produce a mass of nonmotile coccoid elements; do not deposit iron in the sheath.

Type species: *Phragmidiothrix multiseptata* Engler.

NOTES: There is only one species which has not been isolated.

Found on seaweed in polluted wate
the northern Adriatic.

Clonothrix (Clo′ no.thrix) Roze, 1
M p. 274. (J. de Botanique, *10*, 1896, 3
DIFFERENTIATING CHARACTERS: Unice
lar,‡ colorless, rod-shaped organisms, 2
width, arranged in chains within a she
divide by transverse division only
produce at the tip spherical, nonmotile
which are extruded singly or in chains.
sheath becomes heavily impregnated
iron or manganese and becomes wide at
base and tapers toward the tip; attac
by a holdfast; false branching is comm

Type species: *Clonothrix putealis* (Ki
ner 1878) Beger.

NOTES: Only one species is recorded.
has not been isolated.

Found in iron- and manganese-bear
waters.

Caulobacter (Cau.lo.bac′ ter) Hen
and Johnson, 1935. *M p. 213.* (J. Bacteri
29, 1935, 4; also *30*, 1935, 83.)

DIFFERENTIATING CHARACTERS: Unice
lar, colorless, nonsporing, curved, r
shaped organisms, less than 2 μ wide,
tached to a substrate by means of a st
produced as a continuation of the cell
parallel to the long axis of the cell; rep
duction by transverse fission of the cell w
the daughter cell separating from the f
end and developing a single polar flagell
The daughter cell breaks away and
duces a stalk from the end of the cell
which the flagellum is located. *Aerobic;*
erotrophic; Gram-negative.

Type species: *Caulobacter vibrioides*, H
rici and Johnson.

NOTES: One species is described. Cells
0.5 to 1.2 μ by 1.5 to 3.0 μ.

In liquid media growth occurs in cha
teristic rosettes with stalks attached
common holdfast.

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* It is not clear from descriptions whether Crenothrix is a chain of separated cells enclosed in a sheath or a multicellular organism in a sheath. The Comprehensive Key avoids a decision on this point which needs clarification.

† It is not clear whether this species is a multicellular organism or a group of unicellular organisms arranged in a chain. The name suggests multicellularity. The Comprehensive Key avoids the issue which needs clarification.

‡ It is not clear whether this organis
a unicellular form occurring in chains
indicated above, or multicellular like
giatoa. That Roze should have considere
a blue-green alga suggests multicellular
The Comprehensive Key avoids the i
which should be clarified.

ılar, low, convex, smooth, glistening
s are produced on agar.

le is not produced.

ite is not produced from nitrate.

ıcid or gas is produced from carbo-
es.

organism requires riboflavin for
but can utilize ammonia nitrogen.

mal temperature, 30° C.

organism was originally isolated
Lake Alexander, Minnesota. It is
ɔn in tap slime in Brisbane, Australia.
Bowers, Weaver, Grula, and Ed-
J. Bacteriol., *68*, 1954, 194; Hou-
Antonie van Leeuwenhoek. J. Micro-
erol., *21*, 1955, 29; and Kandler *et al.*,
Mikrobiol. *21*, 1954, 57 for more re-
escriptions.

lionella (Gal.li.o.nel′ la) Ehren-
1838. *M p. 214*. (Die Infusionsthier-
1838, 166.)

E: Woutera van Iterson (1958) has
n a monograph entitled *Gallionella*
inea Ehrenberg in a Different light,
hed by N. V. Noord-Hollandsche
vers Maatschappij, Amsterdam, pp.
The monograph was received by the
r too late to be examined in detail, but
ear that it merits very thoughtful con-
ion. In van Iterson's own words (p.

e study has led to the conception
the basic structure of *Gallionella*
nea is the ferruginous band to which
cribed properties of a form of pre-
ly autotrophic life. In or on the bands
riously shaped regions of dense ma-
and in organic continuity with the
inous structures cells may develop.
ells are sometimes flagellated and can
e observed free; many or all of them
pparently capable of a fully hetero-
ic way of life. Apart from this mode of
pment, under some conditions the
s may perpetuate by the formation of
orangia of *Gallionella*' or by means of
rimordial plasm.' Prolonged study of
ruginous ribbons may disclose further
new aspects of fundamental problems connected with life."

Should this study receive confirmation, the insertion in *Bergey's Manual* will be completely outmoded. Readers are therefore asked to treat the *Manual's* descriptions with caution.

DIFFERENTIATING CHARACTERS: Unicellular, colorless, curved, rod-shaped organisms, less than 2 μ wide, which are attached by means of a stalk originating from the concave side of the cell. The stalk, which is ribbon-like and usually twisted, is composed of or impregnated with oxides of iron and dissolves completely in dilute mineral acid.

Type species: *Gallionella ferruginea* Ehrenberg.

NOTES: Five species have been described in the *Manual*. They are curved to rounded cells, 0.6 by 1.2 to 5.0 μ, which secrete a ribbon-like stalk, dumbbell-shaped in cross section from the concave side. The stalk is reputed to be composed solely of ferric hydroxide, although this is doubtful. It is possibly of an organic nature and impregnated with iron. It dissolves completely in dilute mineral acids. The stalk is usually characteristically twisted. This is attributed in the *Manual* to a slow rotatory motion of the cells, a most unlikely suggestion. Van Iterson (1958) has published electron photomicrographs showing polar flagella on most of the cells.

Found in iron bearing waters in cool climates. They have been found in local Brisbane (Australia) waters in the middle of winter. The iron-encrusted stalks minus the terminal cells are quite commonly encountered among the hardened sheaths of *Leptothrix*.

Siderophacus (Si.de.ro′ pha.cus) Beger, 1944. *M p. 216*. (Ber. deut. botan. Ges., *61*, 1944, 12.)

DIFFERENTIATING CHARACTERS: Unicellular, colorless, curved or straight, rod-shaped organisms, less than 2 μ wide, *attached* at right angles to the axis of the cell by means of a horn-shaped stalk, circular in cross section and not twisted. The stalk is impregnated with ferric hydroxide.

Type species: *Siderophacus corneolus* (Dorff) Beger.

NOTES: Only one species has been described. Cells are 0.6 to 1.0 μ by 2.5 to 3.0 μ. The stalks, broader at the base than at the top, are 15 to 30 μ long.

Found in iron-bearing waters.

Nevskia (Nev′ ski.a) Famintzin, 1892. *M p. 216.* (Bull. Acad. Imp. Sci., St. Pétersburg, Sér. IV, *34*, (N. S. 2), 1892, 484.

DIFFERENTIATING CHARACTERS: Unicellular, colorless, rod-shaped organisms, 2 μ wide; attached, at right angles to the long axis of the cell, to the ends of lobose, dichotomously branched stalks composed of gum. Iron is not deposited in the stalk.

Type species: *Nevskia ramosa* Famintzin.

NOTES: A single species has been described. Cells are 2.0 by 6 to 12 μ and contain a number of highly refractile globules of fat or sulfur. Colonies are globular, bush-like or plate-like, and float on the surface of water.

It is possible that there is some relationship between these organisms and those of the genus *Thiobacterium* Janke. Neither has been isolated (author's note).

Pasteuria (Pas.teu′ ri.a) Metschnikoff, 1888. *M p. 279.* (Ann. inst. Pasteur, *2*, 1888, 166.)

DIFFERENTIATING CHARACTERS: Unicellular, colorless, pear-shaped cells 1 to 2 μ wide; *attached* to each other or to a firm substrate by holdfasts secreted at the narrow end; multiplication by longitudinal fission or by terminal budding.

Type species: *Pasteuria ramosa* Metchnikoff.

NOTES: Only one species is described. Cells are 1 to 2 μ by 4 to 5 μ. They form cauliflower-like masses. The cells and method of reproduction resemble those of *Chamaesiphon*, a blue-green alga.

Parasitic on fresh water crustacea (*Daphnia* spp.). Forms resembling them have been found on submerged glass slides.

Blastocaulis (Blas.to.cau′ lis) Henrici and Johnson. *M p. 279.* (J. Bacteriol. 1935, 84.)

DIFFERENTIATING CHARACTERS: Unic lar, colorless, pear-shaped to spherica ganisms, 2 μ in width, borne on the end long slender stalk attached to a hold reproduce by budding from the free en the cell.

Type species: *Blastocaulis sphaerica* rici and Johnson, 1935.

NOTES: There is only a single species scribed. It has not been isolated. Du budding the bud stains intensely and formly whereas the mother cell shows ferentiation. The former is usually Gr positive and the latter Gram-negative. organisms described were from water Lake Alexander, Minnesota, where temperature did not exceed 23° C.

Azotobacter (A.zo.to.bac′ ter) jerinck, 1901. *M p. 283.* (Zentr. Bakte Parasitenk., Abt. II, *7*, 1901, 567.)

DIFFERENTIATING CHARACTERS: F living, unicellular, colorless, nonspor spherical to rod-shaped oranisms wh may exceed 2 μ in width; motile with pe richous flagella or nonmotile; Gram-ne tive; *aerobic*; heterotrophic; fix gase nitrogen in the absence of a legumin host; do not deposit iron about the cell sulfur internally.

Type species: *Azotobacter chroococ* Beijerinck.

NOTES: The genus as described in *Manual* has three species. *A. chroococ* and *A. agilis* are large rods, 2 to 3 μ b to 6 μ. *A. indicus* is much smaller, 0. 1.2 μ by 1.7 to 2.7 μ. Motile by mean peritrichous flagella.

A. chroococcum during the developm of the colonies changes from a unifor staining rod to a lozenge-shaped cell finally to a spherical cell with a man reticulate stain. Colonies during this cha turn from transparent to milky or op cent. Finally the cells deposit a mel pigment and cell walls become consider thickened—the so-called "arthrosp stage—and colonies turn black.

agilis produces slightly yellowish
s with a pigment—not fluorescin—
fluoresces a bluish green under ultra-
light. Cells are nearly spherical.
(Proc. Linnean Soc. N. S. W., *78*,
5) places this organism in the genus
occus.

indicus appears to be bicellular, al-
it is described in the *Manual* as
two highly refractile bodies in the
f a unicellular form. It is very acid-
t.

x (Koninkl. Ned. Akad. Weten-
, Proc. Sect. Sci., *53*, 1950, 145; and
ogoriensis, *1*, 1950, 1) has made this
s the type species of a new genus,
inckia. His opinion is strongly sup-
by Tchan (Proc. Linnean Soc. N.
78, 1953, 85).

three species grow poorly on peptone
owly and copiously on a glucose- or
itol-mineral salts agar, fixing atmos-
nitrogen.

izobium (Rhi.zo′bi.um) Frank,
M p. 285. (Ber. deut. Botan. Ges.,
9, 380.)

FERENTIATING CHARACTERS: Unicellu-
olorless, nonsporing, rod-shaped or-
ns, less than 2 μ wide; motile with
chous flagella* or nonmotile; Gram-
ve; heterotrophic; *aerobic*; capable
ning nodules on, and fixing nitrogen
ociation with, leguminous plants un-
ecified conditions.

e species: *Rhizobium leguminosarum*

ES: Six species have been described.

Differentiation is based on host specificity, and acid formation in, or peptonization of, litmus milk. Some practical results have been achieved by application of serological tests.

In the host plant the bacterial cells undergo pleomorphic changes into T- and Y-shaped "bacteroids" generally considered to be degenerate.

Away from the host they will not fix nitrogen.

Acid production from sugars is very slight.

Milk is peptonized by several host species.

The organisms are easy to identify if isolated from the host. If they are isolated from soil, identification may be exceedingly difficult.

If nodule formation is ignored and species are compared with those of other genera, they show affinity to the genera *Pseudomonas*, *Alcaligenes*, and *Paracolobactrum*.

Agrobacterium (Ag.ro.bac.te′ri.um) Conn, 1942. *M p. 288*. (J. Bacteriol., *44*, 1942, 359.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, nonsporing, straight, rod-shaped organisms, less than 2 μ wide; motile with flagella or nonmotile; Gram-negative; *aerobic*; heterotrophic; acid production from glucose and lactose varies with the species; no gas produced in peptone sugars; five of the seven species are pathogenic to plants and produce hyperplastic diseases.

They *do not or are not known to*: hydrolyze cellulose or agar or chitin, utilize phenols, oxidize thiosulfate autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce alcohol from glucose, or fix gaseous nitrogen.

Type species: *Agrobacterium tumefaciens* (Smith and Townshend) Conn.

NOTES: Rods 0.2 to 0.8 μ by 1 to 3.0 μ. Generally motile although the location of flagella is in doubt. Some cells have a single flagellum in a polar or lateral position. Others have several and are peritrichous.

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eifson and Erdman (Antonie van
enhoek, J. Microbiol. Serol., *24*, 1958,
ve recently examined the flagellation
strains of *Rhizobium* and describe two
of flagellation. In one, flagella are
and subpolar with a wavelength of
2.2 μ. The other type shows peritri-
flagellation with usually one flagellum
s often, several flagella with a wave-
of 1.3 to 1.6 μ. The former grow
on peptone media and the latter
ely rapidly.
view of this report the classification of
oup needs re-examination.

This may vary, as in *Chromobacterium*, with the medium.

Grow well on nutrient agar and frequently also in mineral salts media with a suitable carbon source. Acid production from carbohydrates is very weak and may be overshadowed by ammonia liberated from the peptone. Litmus milk is turned alkaline and is peptonized by some species.

Most species are pathogenic to plants and produce hyperplastic diseases such as galls and hairy root. When isolated directly from soil the organisms may be difficult to identify. They have affinities to the genera *Pseudomonas*, *Alcaligenes*, *Achromobacter*, *Paracolobactrum*, and *Flavobacterium*.

Chromobacterium[*] (Chro.mo.bac.te'ri.um) Bergonzini, 1881. *M p. 292.* (Ann. Soc. Naturalisti in Modéna, Ser. 2, *14*, 1881, 153.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonsporing, nonphotosynthetic, rod-shaped organisms, less than 2 μ wide, with a deep purple color due to the pigment violacein; motile with peritrichous or polar flagella; Gram-negative; heterotrophic.

They *do not or are not known to*: oxidize ethyl alcohol quantitatively to acetic acid, produce ethyl alcohol from glucose, or fix gaseous nitrogen.

Type species: *Chromobacterium violaceum* (Schroeter) Bergonzini.

NOTES: Four species are described. They are differentiated in the *Manual* on cultural characteristics.

Rods, 0.4 to 0.8 μ by 1.0 to 5.0 μ, motile by polar or peritrichous flagella. Gram-negative. Leifson (J. Bacteriol., *71*, 1956, 393) states that two different types of flagella are produced, the polar being quite different from the lateral flagella. Some cultures produce only polar and others produce both.

The organisms grow well on nu
agar, producing a violet pigment wh
not soluble in water. It has been iden
as violacein, chemically related to in
Colorless variants are not uncommon.

Cultures are difficult to maintai
parently because of high sensitivit
peroxides.

Leifson has recently re-examined
strains of purple organisms from d
ent sources and gives the following i
mation.

All strains produced acid from glu
and fructose.

None of them produced acid from
tose, dulcitol, or salicin; none hydrol
urea or produced indole.

Gelatin was liquefied by all but t
strains and nitrates reduced by all but
strain.

Sixteen of the 28 strains fermented
cose, fructose, and inulin and grew at 37

Six oxidized glucose and fructose
several other sugars and did *not* grow
37° C.

The *Manual* records four species,
violaceum, *C. amethystinum*, *C. janthin*
and *C. marismortui*.

Leifson's strains probably cover *C.*
laceum and possibly *C. janthinum* and
new species.

C. marismortui is halophilic.

Alcaligenes (Al.ca.li'ge.nes) Ca
lani and Chalmers, 1919. *M p. 297.* (M
ual Trop. Med., 3rd ed., 1919, 936.)

DIFFERENTIATING CHARACTERS: F
living, unicellular, nonphotosynthetic, n
sporing, straight, rod-shaped bacteria,
than 2 μ wide; do not form zoogloea;
motile or motile with flagella; Gram-r
tive; *aerobic*; heterotrophic; grow we
meat extract agar without the addi
of growth factors; no acid or gas produ
from glucose or lactose; litmus milk tur
strongly alkaline.

They *do not or are not known to*: hydro
agar or cellulose, liquefy Loeffler's ins
sated serum, utilize phenol or alkylam

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[*] The more recent papers by P. H. A. Sneath and his associates (J. Gen. Microbiol., *15*, 1956, 70 and 99; *20*, 1959, 284 and 414) should be consulted for this genus.

ates, oxidize thiosulfate or hydrogen
phically, oxidize ethyl alcohol quan-
ely to acetic acid, produce alcohol
lucose, fix gaseous nitrogen, deposit
r produce water-soluble pigments.
re not halophilic.

e species: *Alcaligenes faecalis* Castel-
d Chalmers.

ES: Six species are described, sepa-
in the *Manual* on motility; gelatin
ction and growth in milk.

ight, rod-shaped, unicellular organ-
.5 to 1.0 μ by 1 to 3 μ. Three of six
s are motile. Although recorded in the
al as peritrichous, other texts record
lagellation. Frequently capsulated.

w quite well on meat infusion agar,
cing translucent grayish colonies or
opaque colonies (depending on spe-
which are frequently viscid with the
lated forms. Gelatin is liquefied by
species.

mus milk is made alkaline and is also
nized by two of the gelatin-liquefying
s.

acid or gas is produced from carbo-
tes.

ole is not produced.

rate reduction is variable.

imal temperature, 20° or 37° C., de-
ng on origin.

nd in the intestinal canal, milk, and
.

romobacter (A.chro.mo.bac′ ter)
y *et al.*, 1923. *M p. 300.* (Bergey's
al of Determinative Bacteriology, 1st
923, 132.)

FERENTIATING CHARACTERS: Free-liv-
unicellular, colorless, nonsporing,
ht, rod-shaped organisms, less than
ide; nonmotile or motile with peritri-
s flagella; Gram-negative; *aerobic*; het-
ophic; grow well on meat infusion agar
or without 3 per cent salt added. No
roduced from carbohydrates; acid is
ced by a few species from glucose or
glucose and lactose; litmus milk acid
changed.

They *do not or are not known to*: hydrolyze agar, cellulose, or chitin; liquefy Loeffler's inspissated serum; utilize alkylamines; oxidize thiosulfate autotrophically; oxidize ethyl alcohol quantitatively to acetic acid; produce ethyl alcohol from glucose; fix gaseous nitrogen; deposit iron; produce any water-soluble pigments; or luminesce.

They are not pathogenic to warm-blooded animals and bear no antigenic relationship, so far as is known, to organisms of the *Enterobacteriaceae.*

Type species: *Achromobacter liquefaciens* (Eisenberg) Bergey *et al.*

NOTES: Fifteen species have been described. They have been separated in the *Manual* on morphological and biochemical properties.

Ten species are motile and have peritrichous flagella.

Colonies are not pigmented but may be translucent to off-white.

Gelatin is liquefied by 8/15 species.

Litmus milk remains unchanged (12/15) or becomes alkaline (1/15) or acid (2/15).

Acid but no gas is produced from glucose by 6/11. Only one species (*A. delmarvae*) produces acid from lactose. No acid or gas is produced by 5/11.

Starch is hydrolyzed by 3/9 species.

Fat is hydrolyzed by 1/3.

Indole is not produced (11/11).

Nitrite is produced from nitrate by 10/14.

Urease is not produced by 4/4 species.

H_2S is produced by 2/7.

Naphthalene is utilized by 2/2 and citrate by 2/3.

Trimethylamine is produced from trimethylamine oxide by 1/7 species.

The organisms are principally of soil or water origin and have an optimal temperature of about 25° C.

Flavobacterium (Fla.vo.bac.te′ ri. um) Bergey *et al.*, 1923. *M p. 309.* (Bergey's Manual of Determinative Bacteriology, 1st ed., 1923, 97.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonsporing, nonphotosynthetic, straight, rod-shaped organisms less than 2 μ wide; nonmotile or motile with peritrichous flagella; Gram-negative; *aerobic*; heterotrophic; with the appropriate salt concentration they all grow on meat infusion and other media and produce colonies with yellow or orange pigments which are not soluble in water; no gas produced from carbohydrates; acid *may* be produced from glucose and lactose; litmus milk may be rendered acid or alkaline or not change. Starch may be hydrolyzed and nitrates may be reduced to nitrite.

They *do not or are not known to*: hydrolyze cellulose, agar, or chitin; utilize phenols or alkylamines; oxidize thiosulfate or hydrogen autotrophically; oxidize ethyl alcohol quantitatively to acetic acid; produce alcohol from glucose; fix gaseous nitrogen; deposit iron; or luminesce.

Type species: *Flavobacterium aquatile* (Frankland and Frankland) Bergey *et al.*

NOTES: Twenty-six species are described. They are separated on motility, pigmentation, action on litmus milk, reduction of nitrate, growth on potato, fermentation of glucose, production of indole, liquefaction of gelatin, and ecological characters.

Straight, rod-shaped, unicellular organisms, 0.5 to 1.0 μ by 0.8 to 3.0 μ. Gram-negative. Fifteen of the 26 recorded species are motile and have peritrichous flagella.

In the presence of the required amount of salt (some species are marine), growth occurs on nutrient agar with the production of an insoluble yellow pigment.

Nineteen out of 26 tested species liquefy gelatin.

Litmus milk remains unchanged or becomes slightly alkaline. Slight acidity is recorded for only two species which do not produce acid from lactose.

Indole is produced by only 1/23 species (*F. suavolens*). Nitrate is reduced by only 9/23.

Acid but *no gas* is produced from glucose by 9/12 species, three of which ferment lactose.

H_2S is produced by 5/14 species.

Urease is not produced (7/7).

Fat is not hydrolyzed (5/5).

Coagulated serum is liquefied by species.

Ammonia is produced from peptone 5/5.

Casein is hydrolyzed by 2/4.

The organisms have been isolated fr water, soil, dairy wastes, sea water, mud, and fish. *F. piscicida* is highly pat genic to fish.

Agarbacterium (A.gar.bac.te′ri. Angst, 1929. *M p. 322.* (Publs. Pu Sound Biol. Sta., Univ. Wash., 7, 1929, 5

DIFFERENTIATING CHARACTERS: Free-l ing, unicellular, nonphotosynthetic, n sporing, straight, rod-shaped organis less than 2 μ wide; nonmotile or motile w peritrichous flagella; Gram-negative; *ae bic*; heterotrophic. With the appropri salt concentration all species will grow meat infusion agar and cause a liquefacti of the agar. The growth is buff, yellow, orange; no gas is produced from carboh drates; acid produced from glucose or la tose by only a few species.

They *do not or are not known to*: hyd lyze cellulose or chitin;* liquefy Loeffle inspissated serum; utilize alginates,* p nols, or alkylamines; oxidize ethyl alco quantitatively to acetic acid; produce al hol in quantities from glucose; fix gase nitrogen or deposit iron. Not pathogenic warm-blooded animals nor antigenic related, so far as is known, to any of *Enterobacteriaceae.*

Type species: *Agarbacterium aurantia* Angst.

NOTES: Twelve species are descri Organisms 0.5 to 1.0 μ by 1 to 4 μ. (species (*A. uliginosum*) is described slightly curved. Six of the twelve recor species are nonmotile. Of the others, t

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* Hydrolysis of alginates and chitin not recorded but probably it occurs.

ritrichous flagella, and for four the
flagellation is not recorded.
in is liquefied by all species except
nator.
is milk remains unchanged except
. *pastinator* which turns it slightly

cid or gas is produced from glucose
ose by 8 of the 12 species. Acid is
ed by *A. pastinator* and *A. uligino-*
m both sugars. Four species are re-
to produce acid from mannitol and
n glucose.
h is hydrolyzed by 8/12 species.
le is not produced (10/10).
is produced by 2/7 species.
en of the 12 species are of marine
The optimal temperature is between
l 28° C.

eckea (Be.neck′e.a) Campbell. *M*
(Bergey's Manual of Determinative
iology, 7th ed., 1957, 328.)
ERENTIATING CHARACTERS: Free-liv-
nicellular, nonsporing, nonphotosyn-
straight, rod-shaped organisms;
with peritrichous flagella; Gram-
ve; *aerobic*; heterotrophic; acid but
produced from glucose; no acid from
; chitin is hydrolyzed; antigenically
herwise unrelated to *Salmonella ty-*

do not or are not known to: hydrolyze
ellulose, or alginates;* utilize phenol;
ethyl alcohol quantitatively to ace-
d; produce alcohol from glucose; fix
s nitrogen, deposit iron; or luminesce.
e species: *Beneckea labra* (Campbell
illiams) Campbell.
ES: Six species are listed. They are
ted in the *Manual* on gelatin lique-
n and production of nitrite from ni-
and acid from sucrose.
organisms grow well on nutrient
Only one of the six species recorded is
nted (yellow-orange).
atin is liquefied by 4/6 species.

here is no record of alginate hydroly-
locally isolated chitin-hydrolyzing
e also hydrolyzed alginate.

Litmus is rendered slightly acid after 2 to 6 days by 4 species, although all fail to produce acid from lactose in peptone water (?).

Acid but no gas is produced from glucose but not from lactose by 6/6 species.

Starch is hydrolyzed by 5/5.

Chitin is hydrolyzed—the significant generic character.

Casein is hydrolyzed by 3/5.

Fat is hydrolyzed by 1/4 species.

Indole is produced by 1/5.

Nitrite is produced from nitrate by 3/6.

Ammonia is produced from peptone by 6/6.

Urease is produced by 1/5.

H_2S is produced by 5/5 species.

Trimethylamine is not produced from trimethylamine oxide (5/5).

All recorded species are of marine origin, principally from sea mud.

The optimal temperature for growth lies between 20° and 30° C.

Escherichia (Esch.er.ich′i.a) Castellani and Chalmers, 1919. *M p. 335.* (Manual Trop. Med., 3rd ed., 1919, 941.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, nonsporing, straight, rod-shaped organisms, less than 2 μ wide; motile with peritrichous flagella or nonmotile; Gram-negative; *aerobic*; heterotrophic; acid and gas produced from glucose and lactose within 48 hours; methyl red-positive; Voges-Proskauer-negative; gelatin liquefied by one species.

They *do not or are not known to*: produce protopectinase; ferment alginates; hydrolyze cellulose or agar; utilize phenol; oxidize thiosulfate autotrophically; oxidize ethyl alcohol quantitatively to acetic acid; produce alcohol from glucose; fix gaseous nitrogen; deposit iron; or produce water-soluble, blue-green or yellow pigments. They are not bioluminescent.

Type species: *Escherichia coli* (Migula) Castellani and Chalmers.

NOTES: Four species are described in the *Manual*. They are differentiated on pigmentation, utilization of citrate, and production of hydrogen sulfide.

Growth occurs readily on meat infusion agar, typical colonies being 2 to 3 mm. in diameter in 24 hours at 37° C; circular, low convex, entire, smooth and glistening, butyrous, and easily emulsified. Colonies are normally an off-white and translucent. Fecal odor.

This type of colony is often called a *coliform* colony. It is characteristic of many Gram-negative rods, particularly those of intestinal origin.

Escherichia aurescens produces a reddish brown pigment which is not prodigiosin (*cf. Serratia*).

The organisms of this genus will use ammonia nitrogen and hence can be grown in a variety of mineral salts media containing a suitable carbon source.

Gelatin is not hydrolyzed except by some strains of *E. freundii.*

Litmus milk is rendered acid and usually clots. The litmus may be reduced.

Nitrites are produced from nitrates.

Indole may be produced.

H_2S may be produced.

Acid and gas are produced from glucose and lactose and many other sugars and sugar alcohols. Acid production from lactose occurs within 48 hours.

Methyl red-positive.

Voges-Proskauer-negative.

Citric acid is utilized as the sole source of carbon by 2/4 species but *not* by the type species.

Catalase-positive.

Facultative anaerobes with a pH range of 4.5 to 8.5 and optimal temperature of approximately 37° C.

Found in the alimentary tract of man and animals and in soil and waters.

Aerobacter (A.e.ro. bac′ ter) Beijerinck, 1900. *M p. 341.* (Zentr. Bakteriol. Parasitenk., Abt. II, *6*, 1900, 193.

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, straight, rod-shaped organisms, less than 2 μ wide; nonmotile or motile with peritrichous flagella; Gram-negative; *aerobic*; h erotrophic; acid and gas produced fr glucose and lactose in 48 hours; meth red-negative; Voges - Proskauer - positi gelatin may or may not be liquefied.

They *do not or are not known to:* produ protopectinase, ferment alginates, hyd lyze agar or cellulose, utilize phenol, o dize thiosulfates autotrophically, produ acetic acid quantitatively from ethyl alc hol, produce alcohol from glucose, depo iron,* or produce any pigments. They a not bioluminescent.

Type species: *Aerobacter aerogen* (Kruse) Beijerinck.

NOTES: Only two species are describ and are separated in the *Manual* on glycer fermentation and gelatin liquefaction.

Straight, rod-shaped, unicellular orga isms. Nonmotile, or motile with perit chous flagella. Gram-negative. Species a frequently capsulated, particularly whe grown at 25° C. in the presence of fermen able carbohydrates.

Grow well on meat infusion agar and al in mineral media containing ammoniu sulfate and a suitable carbon source. Col nies are usually more opaque than those *Escherichia* and are mucoid with the ca sulated types.

Gelatin is liquefied slowly by *A. cloac* but not by *A. aerogenes.*

Litmus milk is acidified and coagulat It is slowly peptonized by *A. cloacae.*

Nitrite is produced from nitrates.

Indole production is variable.

Acid and gas are produced from gluc and lactose and several other sugar al hols. Acid production from lactose occ within *48 hours.*

Methyl red-negative.

Voges-Proskauer-positive.

Citrate is used as the sole source carbon.

Catalase-positive.

---

* Iron may be deposited from the fer ammonium citrate medium as a result metabolism of the citrate, but this is recorded in the *Manual.*

ltative anaerobes with a pH range oximately 4.5 to 8.5.

optimal temperature is between 30° ° C.

ıd mainly on grain and plant tissue feces, soil, and water.

e have been repeated and well sub- ted reports of nitrogen fixation by *cter.*

Manual differentiates between the *Aerobacter* and *Klebsiella* on the basis sule formation and pathogenicity of ter. These are not valid criteria.

Enterobacteriaceae Subcommittee Nomenclature Committee of the ational Association of Microbiolo- as grouped the two genera under the siella-aerogenes Group" (see Intern. of Bacteriol. Nomen. and Taxon., *4,*

bsiella (Kleb.si.el′ la) Trevisan, *M p. 344.* (Atti accad. Fisio-Medico- ica in Milano, Ser. 4, *3*, 1885, 105.)

FERENTIATING CHARACTERS: Free-liv- unicellular, colorless, nonsporing, ht, rod-shaped organisms less than 2 e; capsulated; nonmotile; Gram-nega- heterotrophic; grow well on meat in- agar; acid and gas usually produced glucose; acid usually produced from e in 48 hours; methyl red-negative; -Proskauer-positive. Virulent forms genic, producing diseases principally piratory origin.

y do not or are not known to: hydrolyze se, agar, or starch; liquefy gelatin; phenol; oxidize thiosulfate auto- ically; oxidize ethyl alcohol quantita- to acetic acid; produce alcohol from se; fix gaseous nitrogen; deposit iron; minesce.

pe species: *Klebsiella pneumoniae* oeter) Trevisan.

TES: Three species are described. They parated in the *Manual* on pathogenic- nd action on glucose. Rod-shaped organisms, 0.3 to 0.5 μ by 2 to 5 μ.

psule formation is a variable character noncapsulated avirulent forms resemble *Aerobacter*. In addition, some *Aerobacter* strains are virulent and some are capsulated.

Colonies produced are often slimy as a result of capsule formation.

Nitrite is produced from nitrate.

H_2S is not produced.

Acid is produced from glucose.

Gas is produced also by 2/3 species.

Acid and gas from glucose and acid from lactose are normal for the group.

Lactose may or may not be fermented and may, if fermented, be fermented very slowly.

Litmus milk becomes acid without coagulation or remains unchanged.

The methyl red and Voges-Proskauer tests vary with the species.

Citrate is utilized by 1/3 species.

The organisms are aerobic and facultatively anaerobic. The optimal temperature is 37° C.

They are pathogenic and have been isolated from cases of pneumonia and other infections of the respiratory tract.

The Enterobacteriaceae Subcommittee has recommended the grouping of both genera together (see notes to *Aerobacter*).

Paracolobactrum (Pa.ra.co.lo.bac′-trum) Borman *et al.*, 1944. *M p. 346.* (J. Bacteriol., *48*, 1944, 361.)

DIFFERENTIATING CHARACTERS: It is possible to frame only a quasidefinition of this genus. The organisms are related biochemically to most of the recognized genera of the *Enterobacteriaceae.* They are colorless, unicellular, nonsporing, Gram-negative, *aerobic*, heterotrophic organisms which produce acid or acid and gas from glucose. Lactose fermentation is usually delayed.

Relationships to other genera and to the various groups into which they are placed by the Enterobacteriaceae Subcommittee of the Nomenclature Committee of the International Association of Microbiologists (indicated by Rome, 1953) are given in the following abbreviated Keys, abstracted from the Comprehensive Key.

The Enterobacteriaceae Subcommittee

in its report to the Nomenclature Committee at the Seventh International Congress of Microbiology at Stockholm (1958) recommended that the genus *Paracolobactrum* be not recognized.

Type species: *Paracolobactrum aerogenoides* Borman *et al.*

Lactose-fermenting motile strains

A. Agar not digested; motile with peritrichous flagella; acid and gas from glucose; gelatin liquefied; protopectinase not produced.

43. Lactose fermented in 2 days...... **44**

Lactose fermentation delayed

Paracolobactrum

(*P. arizonae* and *P. aerogenoides* strains 4611, 1721, and 19111 ... and Arizona Group, Rome, 1953.)

44. Methyl red-positive; Voges-Proskauer-positive or -negative.......... **45**

Methyl red-negative; Voges-Proskauer-positive..................... **Aerobacter**

45. No gas produced from lactose in 2 days

Paracolobactrum

(*P. intermedium* strains 8011, 13311, and 11411.)

Gas produced from lactose within 2 days

Escherichia

(*E. freundii*; see also Arizona Group, Rome, 1953.)

B. Agar not digested; motile with peritrichous flagella; gelatin not liquefied; nitrogen not fixed; alginates not fermented; methyl red-positive; Voges-Proskauer-negative.

49. Lactose fermented within 2 days.. **50**

Lactose fermentation delayed

Paracolobactrum

P. coliforme strains 28221, 5511, 6611, 2611, 31611, 111, 15411, 1811, 33811, and 4361.

P. intermedium strains 14011, 12611, and 1421.

Bethesda-Ballerup Group, Rome, 1953, for gas-producing strains with slow lactose fermentation.

50. Acid and gas from lactose

Escheri

(Escherichia, Rome, 1953 for m strains.)

Acid only from lactose

Paracolobact

(*P. intermedium* strains 14011 12611.)

C. Agar not digested; motile with pe richous flagella; gelatin not liquefied; ni gen not fixed; alginates not ferment methyl red-negative; Voges-Proskauer- itive.

51. Lactose fermented in 2 days

Aeroba

Lactose fermentation delayed

Paracolobactr

(*P. aerogenoides* strains 721, 37711, 37211.)

D. Agar not digested; motile with pe richous flagella; acid only from gluc cellulose not hydrolyzed; colorless coloni

58. Flagella peritrichous

Paracolobactr

(Anaerogenic strains and also the anae genic strains of the Bethesda-Baller Group, Rome, 1953, which ferment l tose slowly.)

Lactose - fermenting nonmot strains

A. Agar not digested; nonmotile; and gas from glucose; not luminesce methyl red-positive; Voges-Proskauer-n ative.

62. Lactose fermented in 2 days

Escheric

(Escherichia, Rome, 1953, for nonmot strains.)

Lactose fermentation delayed

Paracolobactr

(*P. coliforme* strains 311, 17611, 16911.)

B. Agar not digested; nonmotile; and gas from glucose; not luminesce methyl red-negative; Voges-Proskauer-p itive.

63. Encapsulated organisms; pathoge causing infections in man and anim

lly of the respiratory tract
Klebsiella

above............ **Aerobacter**
siella - Aerogenes Group, Rome,
.)

gar not digested; nonmotile; acid
om glucose; grow well on meat in-
agar; colorless colonies; cellulose not
zed.

ctose fermented in 2 days...... **69**

fermentation delayed; enteric patho-
.... **Shigella**
gella, Rome, 1953, slow lactose fer-
ters.)

capsulated; pathogenic
Klebsiella

s above.........**Achromobacter**

se-nonfermenting strains

Nonmotile; acid and gas from glu-
not agglutinable with Shigella
nella antisera; not luminescent; Vo-
oskauer-positive.

rganisms encapsulated; cause respira-
nd other infections in man
Klebsiella

as above..... **Paracolobactrum**
aerogenoides strains 32011 and
11.)

Motile with peritrichous flagella; acid
s from glucose; colorless colonies; not
escent.

Gelatin liquefied *or* indole produced
th; organisms may show a marked
ling growth on solidified agar with a
f moisture on the surface. **Proteus**
also *Paracolobactrum coliforme*
ins 4361 and 33811 and Providence
up, Rome, 1953, for aerogenic
ins.)

te: Proteus spp. are probably also
e-positive. The character is not men-
d in the descriptions in the *Manual.*

in is not liquefied and indole is not
ced; colonies do not spread on solidi-
gar.

Voges-Proskauer-positive
Paracolobactrum
aerogenoides strains 32011, 37711,
11, 37511, and 32821.)

Voges-Proskauer-negative.. **Salmonella**
(Salmonella Group, Rome, 1953.) (See also *Paracolobactrum intermedium* strains 12611 and 1421 and non-lactose-fermenting strains of the Bethesda-Ballerup Group, Rome, 1953.)

Alginobacter (Al.gi′no.bac.ter) Thjøtta and Kåss, 1945. *M p. 348.* (Avhandl. Norske Videnskaps-Akad., Oslo. I Mat.-Naturv. Kl., No. 5, 1945, 17.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, straight, rod-shaped organisms; motile with peritrichous flagella; Gram-negative; *aerobic*; heterotrophic; acid and gas are produced from glucose and lactose; gelatin not liquefied; *alginates fermented with the production of acid and gas;* hydrogen sulfide produced; methyl red-positive; Voges-Proskauer-weak; citrate utilized; nitrite produced from nitrate.

It *does not or is not known to*: hydrolyze agar or cellulose, utilize phenol, oxidize thiosulfate autotrophically, oxidize ethyl alcohol to acetic acid quantitatively, produce ethyl alcohol from glucose, fix gaseous nitrogen, or deposit iron.*

Type species: *Alginobacter acidofaciens* Kåss, Lid, and Molland.

NOTES: There is only one species; found in soil. Optimal temperature, 30° C.

Erwinia (Er.wi′ni.a) Winslow *et al.*, 1917. *M p. 349.* (J. Bacteriol., *2*, 1917, 560.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, nonsporing, straight, rod-shaped organisms less than 2 μ wide; motile with peritrichous flagella. Gram-negative (except for two species); *aerobic*, heterotrophic; produce acid or acid and gas from glucose; produce wilts and necrotic diseases of plants.

They *do not or are not known to*: hydrolyze agar, cellulose, or chitin; utilize phenol or alkylamines; oxidize ethyl alcohol quantitatively to acetic acid; oxidize thiosulfate autotrophically; produce alcohol from glucose; fix atmospheric nitrogen or deposit iron.

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* Since this organism metabolizes citrate there is a possibility that it will deposit iron from ferric ammonium citrate media.

Species differ considerably on other characters, of which the more commonly described are presented in the Notes.

Type species: *Erwinia amylovora* (Burrill) Winslow *et al.*

NOTES: Seventeen species are recorded. They are separated in the *Manual* on the type of lesion produced; color; hydrolysis of starch; coagulation of milk; utilization of ethyl alcohol, dulcitol, malonate, and hippurate; gelatin liquefaction and growth in Uschinsky's solution.

Rods 0.4 to 0.9 μ by 0.8 to 2.5 μ. Fifteen out of seventeen species are Gram-negative. Two (*E. carnegieana* and *E. citrimaculans*) are described variously as Gram-positive and Gram-negative.

Grow well on meat infusion agar, producing colonies which are a grayish white, yellowish, brown, or red (*Erwinia rhapontica*). The red pigment is not prodigiosin.

Gelatin is liquefied by 13/17 species.

Starch is hydrolyzed by 2/15 species.

Blood serum is not liquefied by species tested on it.

Indole is produced by three species.

H_2S is produced by 7/13 species.

Nitrite is produced from nitrates by 15/17.

Citrate is utilized by all of four species tested.

Acid is produced from glucose by all species. Gas is produced by *E. chrysanthemi*, *E. carnegieana*, *E. dissolvens*, *E. nimipressuralis*, *E. carotovora*, and *E. atroseptica*.

E. carotovora produces acid from 5 per cent ethyl alcohol.

Acid is produced from lactose by 13 of the 16 species for which it is recorded. Gas is also produced by the species listed under glucose.

Litmus milk is turned acid and clotted by most lactose fermenters.

The methyl red and Voges-Proskauer reactions are not recorded for all species. Of the nine recorded eight are methyl red-negative and Voges-Proskauer-positive. *carotovora* is methyl red-positive and Vo
Proskauer-negative.

Species are noted for the productio
diseases in plants. Six species, all of wh
produce acid but no gas from glucose,
responsible for dry rots, galls, and wilts
plants but *not* soft rot. All other spe
are responsible for the production of s
rots of various plants. The soft rot is cau
by the breakdown of the middle lamella
the plant parenchyma by the enzyme pro
pectinase.

Serratia (Ser.ra′ti.a) Bizio, 1823.
p. 359. (Polenta porporina, Biblioteca i
o sia Giorn. lettera, sci., arti, *30*, 1823, 28

DIFFERENTIATING CHARACTERS: Free-li
ing, unicellular, nonphotosynthetic, ro
shaped organisms less than 2 μ wide; mot
with peritrichous flagella; Gram-negati
aerobic; heterotrophic; grow well on me
infusion agar *producing a red pigment*, *pr*
digiosin, which is insoluble in water.

They *do not or are not known to*: hydr
lyze agar or cellulose, utilize phenol
alkylamines, oxidize ethyl alcohol quan
tatively to acetic acid, produce alcohol
quantity from glucose, fix atmospheric n
trogen, deposit iron, or luminesce.

Type species: *Serratia marcescens* Bizio

NOTES: Five species are described. Th
are separated in the *Manual* on solubil
of the pigment in water and alcohol; p
duction of gas from glucose; production
acetoin; and growth in broth.

Very small organisms, 0.5 by 0.6 to 2.0

Grow well on meat infusion agar. Co
nies on various media are rose red to a de
magenta red because of the production
the pigment, prodigiosin, which disti
guishes these organisms from other r
pigmented bacteria. The pigment is read
soluble in alcohol and some other orga
solvents but not in water.

Pigment production is variable and
merous colorless mutants are produced;
some instances pigmentation is complet
lost.

Gelatin is liquefied by all species.

us milk is rendered acid and coagu-
.
is produced from glucose and lac-
as is produced but may not always
le because of solubility.
s-Proskauer positive for 3/5 species.
ates are reduced to nitrites.
ethylamine is produced by most
.
bic and facultatively anaerobic.
optimal temperature is between 25°
° C.
d in water, soil, and milk.

eus (Pro′ te.us) Hauser, 1885. *M*
(Manual Trop. Med., 3rd ed., 1919,

ERENTIATING CHARACTERS: Free-liv-
unicellular, colorless, nonsporing,
t, rod-shaped organisms, less than 2
; motile with peritrichous flagella;
- negative; *aerobic*; heterotrophic;
well and often produce a swarming
on meat infusion agar; acid and gas
ed from glucose. No acid produced
lactose; gelatin liquefied *or* indole
ed *or* both; urease-positive.

y do not or are not known to: hydrolyze
se, utilize phenol, oxidize thiosulfate
ophically, oxidize ethyl alcohol quan-
ely to acetic acid, produce alcohol
tatively from glucose, fix gaseous
n, or deposit iron.

e species: *Proteus vulgaris* Hauser.

ES: Five species are described in the
l. Rods 0.5 to 1.0 μ by 1 to 3 μ.
w well on meat infusion agar, usually
thin moist spreading colony which
ntly covers the whole plate.
atin is liquefied by 2/5 species.
ole is produced by 4/5 species.
mus milk usually becomes alkaline.
d and gas are produced from glucose.
tose is not fermented.
rite is produced from nitrate.
rate is utilized by 4/5 species.
species produce phenyl pyruvic acid
phenylalanine.
imal temperature, 37° C.
nd in putrefying materials and often associated with intestinal disorders, especially in children, and in urinary tract infections.

Strains of the type species *Proteus vulgaris* have been found to have antigenic characters similar to the *Rickettsia* of typhus fever.

Salmonella (Sal.mo.nel′ la) Lignières, 1900. *M p. 368.* (Rec. méd. vétér., Sér. 8, 7, 1900, 389.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, straight, rod-shaped organisms, less than 2 μ wide; motile with peritrichous flagella or nonmotile (*S. pullorum* and S. *gallinarum*); Gram-negative; *aerobic*; heterotrophic; grow well on meat infusion agar, producing discrete, colorless colonies on firm media; produce acid and gas from glucose (except *S. typhosa* and *S. gallinarum*); no acid from lactose; Voges-Proskauer-negative; gelatin not liquefied; indole not produced; urease-negative.

Pathogenic; contain antigens assigned to the genus *Salmonella* (see Intern. Bull. of Bacteriol. Nomen. and Taxon., *4*, 1953).

They *do not or are not known to:* hydrolyze cellulose, utilize phenol, oxidize thiosulfate autotrophically, oxidize ethyl alcohol quantitatively to acetic acid, produce alcohol from glucose, fix gaseous nitrogen, deposit iron, or luminesce. They are not halophilic.

Type species: *Salmonella choleraesuis* (Smith) Weldin.

NOTES: These represent only commonly encountered species. The genus as presented in the *Manual* has 10 described species.

Collectively, there are 343* serotypes separated into groups on the basis of the smooth, somatic antigens and serotypes on the flagellar antigens.

In addition to the differentiating characteristics, the following should be noted.

* This number has been very greatly increased. The latest report is not yet available but it is believed that the figure exceeds 500 (1959).

Litmus milk turns slightly alkaline or remains unchanged.

H_2S production is variable.

Acid and gas are produced from glucose by all but two important species, *S. typhosa* and *S. gallinarum* which produce only acid. *Lactose*, *sucrose*, and *salicin* are not fermented.

Nitrite is produced from nitrate.

Citrate is utilized by most species.

Optimal temperature 37° C.

All species cause disease in man or animals, usually of an alimentary nature. *S. typhosa* causes typhoid fever. *S. paratyphi* and *S. schottmuelleri* cause typhoid-like fevers. Other species cause gastroenteritis.

Shigella (Shi.gel′la) Castellani and Chalmers, 1919. *M p. 384.* (Manual Trop. Med., 3rd ed., 1919, 936.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, straight, rod-shaped organisms, less than 2 μ wide; nonmotile; Gram-negative; *aerobic*; heterotrophic; grow well on meat infusion agar, producing discrete colonies; acid but no gas is produced from glucose; no acid from lactose or lactose fermentation delayed. Enteric pathogens which agglutinate with *Shigella* antisera. (See Intern. Bull. of Bacteriol. Nomen. and Taxon., *4*, 1953.)

They *do not or are not known to*: hydrolyze agar or cellulose, utilize phenol, oxidize thiosulfate autotrophically; oxidize ethyl alcohol quantitatively to acetic acid, produce ethyl alcohol in quantity from glucose, fix gaseous nitrogen, or deposit iron.

Type species: *Shigella dysenteriae* (Shiga, 1898) Castellani and Chalmers, 1919.

NOTES: Eight species are described. They are separated on the production of acid from mannitol, lactose, arabinose, rhamnose, xylose, and dulcitol; production of indole and trimethylamine and on serological reactions.

Gelatin is not liquefied.

H_2S is not produced.

Nitrite is produced from nitrate.

Litmus milk turns alkaline except wi the slow lactose-fermenting *Shigella disp* and *Shigella sonnei*.

Indole production is variable.

Optimal temperature 37° C.

Cause of enteric bacillary dysentery man.

Bacteroides (Bac.te.ro.i′des) Cast lani and Chalmers, 1919. *M p. 424.* (Ma ual Trop. Med., 3rd ed., 1919, 959.)

DIFFERENTIATING CHARACTERS: Free-li ing, unicellular, nonphotosynthetic, no sporing, rod-shaped organisms, less th 2 μ wide; nonmotile or motile with flagell Gram-negative; *anaerobic*; heterotrophi do not ferment glucose with the producti of large quantities of ethyl alcohol or fe ment acetate and *n*-butyrate with t production of methane; do not requi sterile fresh tissue or ascitic fluid f growth.

Further characters which provide a un form differentiation of the genus *Bacteroid* from other genera of anaerobic, Gram-neg tive rods are lacking. The separation *Bacteroides* from *Fusobacterium* on the bas of the shape of cells (the former, round ended and the latter, pointed) is not su ported by the descriptions of the specie Similarly differentiation from other gener on the basis of pleomorphism is not su ported by the species descriptions. T author has attempted a separation at t species level in the keys.

Type species: *Bacteroides fragilis* (Veill and Zuber) Castellani and Chalmers.

NOTES: Thirty species are describe They are differentiated on morphologi and biochemical characters.

Rod-shaped organisms, pleomorphic old cultures, 0.5 to 1.0 μ by 2 to 6 μ. On 2/30 species are motile and have peri chous flagella. Gram-negative.

The majority of species will grow on me infusion agar. Two are favored by the ad tion of ascitic fluid. One species is hal philic. Colony types vary with the speci

Gelatin is liquefied by 10/28 species.

Litmus milk is turned acid and may

ated by 16/26. It is peptonized by

uction of nitrite from nitrate is
re for all species in which it is re-
(16).

le is produced by 9/19 species.

is produced by 5/23 species from pep-
ter without added carbohydrates.

or acid and gas is produced from
by 24/27 species. Acid is produced
ıctose by 20/27.

is produced by 15/22 species only.

gulated serum is liquefied by 1/9.

mal temperature, 35° to 37° C.

nd in the alimentary canal and buccal
of man and animals, in abscesses and
posing organic matter.

obacterium (Fu.so.bac.te′ ri.um)
, 1922. *M p. 436.* (Zentr. Bakteriol.
tenk., Abt. I, Orig., *87*, 1922, 536.)

ERENTIATING CHARACTERS: Free-liv-
nicellular, colorless, nonsporing, rod-
l organisms with pointed ends, less
2 μ wide; nonmotile or motile with
ı; Gram-negative; *anaerobic;* hetero-
c; acid but no gas produced from
e; variable reaction on lactose; do not
ce large quantities of ethyl alcohol
glucose or methane from acetate and
rate.

e species: *Fusobacterium fusiforme*
n and Zuber) Hoffmann.

ES: Six species are described in the
ıl. They are spindle shaped rods with
d ends, straight or slightly curved.
one species is motile (*F. preacutum*).
negative. Two species are 8 to 16 μ
The remaining four do not exceed 4 μ.
will not pass through Chamberland
ers.

wth of two species is favored by
or serum. Others grow well on meat
on agar but not on plain peptone

atin is not liquefied.

nus milk is not altered.

ole is not produced.

rite is produced by 1/6 species.

d is produced from glucose by all species. Three also produce acid from lactose.

No gas is produced from carbohydrates or from peptone.

Optimal temperature, 37° C.

Found in the buccal cavity and intestinal tract of man and animals.

An excellent method for the isolation of oral *Fusobacteria* is described by Omata and Disraely, J. Bacteriol., *72*, 677.

Dialister (Di.a.lis′ ter) Bergey *et al.*, 1923. *M p. 440.* (Bergey's Manual of Determinative Bacteriology, 1st ed., 1923, 271.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, rod-shaped organisms of such small size that they are capable of passing through the Chamberland L2 filter; nonmotile; Gram-negative; *anaerobic*; heterotrophic; acid but no gas produced from glucose; lactose not fermented; found in the upper respiratory tract.

Type species: *Dialister pneumosintes* (Olitsky and Gates) Bergey *et al.*

NOTES: Two species are described. Very minute rods, 0.15 to 0.3 μ by 0.5 to 1.0 μ.

The type species will not grow on meat infusion agar but requires the addition of blood on fresh tissue fluids.

D. granuliformans grows on meat infusion agar.

Colonies are very small and colorless.

Litmus milk is not changed.

Indole is not produced.

Nitrite is not produced.

Acid but no gas is produced from glucose but not from lactose.

Optimal temperature 37° C.

Found in the respiratory tract of man.

Sphaerophorus (Sphae.ro′ pho.rus) Prévot, 1938. *M p. 441.* (Ann. inst. Pasteur, *60*, 1938, 297.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing organisms which may exhibit a marked degree of pleomorphism, the pleomorphic forms frequently exceeding 2 μ in width and occasionally branched; nonmotile (17/18) or motile with flagella (1/18); Gram-negative; *anaerobic;* heterotrophic; do not produce ethyl alcohol from glucose or methane from acetate or *n*-butyrate; variable reactions on glucose and lactose.

Characters which will further differentiate the genus as a whole from other anaerobic Gram-negative rods are lacking. The author has attempted differentiation at the species level.

Type species: *Sphaerophorus necrophorus* (Flügge) Prévot.

NOTES: Eighteen species are described. They are differentiated in the *Manual* on morphological, nutritional, and biochemical characteristics.

Straight or curved, rod-shaped organisms showing very marked pleomorphism. Filamentous and branching forms occur. All cells except swollen pleomorphic ones are less than 2 μ wide.

Only 1/18 species, *S. bullosus,* is motile and has peritrichous flagella.

Serum or ascitic fluid is required by seven species.

Coagulated serum or egg is not liquefied by 6/6.

Gelatin is slowly liquefied by only 2/18 species.

Litmus milk is acidified by 5/17 species and is peptonized by 2 only.

Indole is produced by 8/12 species.

H_2S is produced by 9/11.

Nitrite is produced from nitrate by 0/8.

Gas is produced from peptone by 8/13 species.

Acid or acid and gas is produced from glucose by 11/13 species. Lactose is fermented by five.

Found in lesions in all parts of the human body particularly where secondary invaders can establish the anaerobic conditions. Found also in the alimentary and urogenital canals.

Streptobacillus (Strep.to.ba.cil′lus) Levaditi *et al.*, 1925. *M p. 451.* (Compt. rend., *180*, 1925, 1188.)

DIFFERENTIATING CHARACTERS: Free- ing, unicellular, colorless, nonsporing, r shaped organisms, normally less than 2 wide and occurring in chains up to 100 150 μ long; may exhibit a high degree pleomorphism, particularly on primary i lation; homogeneous in young cultures b become granulated, beaded, and swollen old cultures and tend to fragment into ro and cocci; large spherical swellings pack with granules are common; choleste globules appear among the growth. pathological material only rods and sho filaments are observed; nonmotile; Gran negative; *aerobic*; heterotrophic; requi blood or ascitic fluid for growth; acid b no gas produced from glucose; variable ac production from lactose; highly virulent f mice causing polyarthritis.

It *does not or is not known to*: hydroly agar or cellulose, utilize phenol, oxidi thiosulfate autotrophically, oxidize eth alcohol quantitatively to acetic acid, pr duce alcohol from glucose, fix gaseous n trogen, or deposit iron.

Type species: *Streptobacillus monilifo mis* Levaditi *et al.*

NOTES: Only one species is described the *Manual.*

Pleomorphic, rod-shaped organism; h mogeneous in young cultures; 0.1 to 0.5 by 2.0 μ; pleomorphic forms may be fi mentous and bulbous and measure up 15 μ across. The spherical swellings a frequently packed with granules. Chol terol globules appear among the grow In pathological material only rods a short filaments occur; nonmotile; Gra negative.

The organism will not grow on m infusion agar but requires ascitic fluid blood. Colonies are 1 to 2.5 mm. in dia ter in 3 days on ascitic fluid; circular, l convex, and colorless. L-phase varia are common.

Gelatin is not liquefied.

Litmus milk is not altered.

Indole-negative.

ite is not produced from nitrate.
-positive.
hylene blue is reduced.
ıse-, catalase-, and oxidase-negative.
l is produced from glucose and other
ıydrates. Lactose fermentation varia-

imal temperature 35° to 38° C.
nly virulent for mice; an intravenous
on causes sepsis and death in 24 to
urs or a chronic disease characterized
rulent polyarthritis, anemia, emacia-
liarrhea, conjunctivitis, and transient
manent paralysis.
ses Haverhill fever in man.

rococcus (Mi.cro.coc′ cus) Cohn,
M p. 455. (Beitr. Biol. Pflanz., *1*,
2, 1872, 153.)

FERENTIATING CHARACTERS: Free-
, unicellular, spherical, nonsporing,
otosynthetic organisms; occasionally
l 2 μ in diameter; arranged singly, in
and in irregular clusters from both
and solid media; *aerobic*; hetero-
ic.

ey *do not* produce at least 0.5 to 0.7
ent of *optically inactive lactic acid* in
extract-glucose-tryptone-phosphate
lowering the pH below 4.0; deposit
of iron on the cells; deposit sulfur
ally or ferment formate with the
ction of methane.

e species: *Micrococcus luteus* (Schroe-
Cohn.

ES: Considerable difficulty may be
ntered in differentiating between the
Micrococcus and the genera *Staphylo-*
and *Pediococcus.*

e genus *Micrococcus*, as described in
venth edition of the *Manual*, includes
ical, nonphotosynthetic, unicellular
nisms which aggregate in irregular
ers and are capable of growth in de-
media in which the sole nitrogen
e is ammonium phosphate. Their acid
ction from carbohydrates is con-
d to be *oxidative*, although there is
ng in the descriptions given in the
Manual to support the latter statement; and the ability to use ammonia nitrogen is cited for only 12 of the 16 species and of these, five are recorded as negative!

The genus *Staphylococcus*, to which only two species, *S. aureus* and *S. epidermidis*, have been allocated, consists of spherical, nonphotosynthetic, unicellular organisms which aggregate in irregular clusters and are dependent on amino nitrogen for growth. Acid production from glucose is considered to be *fermentative* but again supporting evidence in the *Manual* appears to be lacking.

The genus *Pediococcus*, which is placed in an entirely different family in the *Manual*, is prone to produce pairs and chains of cells but forms tetrads regularly in acid media. Its species produce a strong acid reaction in sugar solutions. The lactic acid produced is optically inactive. The titratable acidity is equivalent to at least 0.5 per cent lactic acid and the pH falls as low as 4.0.

The use of a quantitative character such as the percentage of acid produced is not a sound taxonomic practice. Inevitably, weak acid-producing strains will be encountered. The optical inactivity of the lactic acid produced is the more important character. Unfortunately there is little information on the nature of the byproducts produced by the genus *Staphylococcus* and the genus *Micrococcus.*

The foregoing points should be borne in mind when considering the differentiating characteristics for the three genera as they appear in the *Manual.*

Williams, Hirch, and Cowan (*vide infra*) have also proposed the separation of a fourth genus, *Aerococcus*, mainly on the basis of its tolerance of 40 per cent bile and 1:400,000 crystal violet. The genus is not listed in the *Manual.*

The following additional notes on the genus *Micrococcus* cover only those tests which have been uniformly applied over all the listed species. Other characters such as chitin hydrolysis, starch hydrolysis, and deamination of urea have been used in the species keys but have not been uniformly applied to all species.

The majority of the 16 species in the genus *Micrococcus* are Gram-positive, although Gram-negative species have been described.

Four species have been reported as motile. They have single flagella or are peritrichously flagellated.

All species grow well on meat infusion agar, although two (*M. morrhuae* and *M. halodenitrificans*) require added salt. Colonies are usually opaque and frequently pigmented white, yellow, orange, pink, or red.

Gelatin is liquefied by 8/16 species.

Growth on liquid media is uniformly turbid.

Acid but no gas is produced from carbohydrates.

Litmus milk is rendered acid by 9/16 species, four of which also peptonize it.

Indole is not produced.

Nitrite is produced from nitrate by 8/16 species and two reduce it to *nitrogen*.

The optimal temperature is 20° to 28° C.

The organisms occur mainly in soil, water, milk, and other foodstuffs. Marine species are isolated from fish.

Staphylococcus (Staph.y.lo.coc′ cus) Rosenbach, 1884. *M p. 464.* (Mikroorganismen bei den Wundinfektionskrankheiten des Menschen, Weisbaden, 1884, 27.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, spherical, nonsporing, nonphotosynthetic organisms; occasionally exceed 2 μ in diameter; arranged singly, in pairs, and in irregular clusters from both liquid and solid media; *aerobic*; heterotrophic.

They *do not* produce at least 0.5 to 0.7 per cent of *optically inactive lactic acid* in yeast-extract glucose tryptone phosphate broth lowering the pH below 4.0; deposit oxides of iron on the cells; deposit sulfur internally or ferment formate with th production of methane.

Type species: *Staphylococcus aureu* Rosenbach.

NOTES: There are only two species recognized in the genus. Both are cocci, 0.8 t 1.0 μ in diameter, occurring in grape-lik clusters. Gram-positive and nonmotile.

They will not utilize ammonia nitroge but grow quite well on meat infusion aga producing circular, entire, low conve smooth, shining, butyrous, opaque colonie They may be white or pigmented a golde yellow. Catalase-positive.

Gelatin is liquefied.

Growth in broth is uniformly turbid.

Litmus milk is acidified and may coagulate.

Acid only is produced from a range o carbohydrates including lactose.

Indole is not produced.

Nitrite is produced from nitrate.

The optimal temperature is 37° C.

Aerobic, facultatively anaerobic.

Staphylococcus aureus is the commo cause of boils, carbuncles, and variou other purulent diseases. Colonies are usually a golden yellow but white variant appear. The pathogenic strains are able t coagulate blood plasma (coagulase-positive).

S. epidermidis, a white form, is a comm inhabitant of human skin.

See also notes on *Micrococcus*.

Aerococcus (Aer.o.coc′ cus) William Hirch, and Cowan. (J. Gen. Microbiol., 1953, 475.)

The above authors give the followi constant generic characteristics.

Gram-positive, nonmotile cocci occurri in pairs or irregular clusters, usually sma Aerobic and facultatively anaerobi growth occurs on solid media at 22° almost as well as at 37° but not at 4 Colonies on blood agar incubated aero cally at 37° for 18 to 24 hours are sem transparent and surrounded by a zone green color. Growth is not inhibited by per cent bile, nor by 1:400,000 crys

. Arginine is not hydrolyzed. The isms survive heating to 60° C. for 30 tes and grow at pH 9.6. Acid and amide extracts do not react with sera red against Lancefield Group D ococci.

pe species: *Aerococcus viridans*.

TE: The organism is a common con- nant from laboratory air.

ffkya (Gaff′ ky.a) Trevisan, 1885. *466*. (Atti accad. Fisio-Medico-Sta- a in Milano, Ser. 4, *3*, 1885, 106.)

FERENTIATING CHARACTERS: Unicellu- olorless, nonsporing, spherical organ- less than 2 μ in diameter; characteris- y arranged in tetrads and encapsulated dy fluids but occurring in pairs, tetrads irregular masses in culture media; otrophic; Gram-positive; *aerobic*.

ey *do not or are not known to*: produce ast 0.5 to 0.7 per cent optically inac- lactic acid in yeast extract-glucose- tone-phosphate broth, lowering the pH v 4.0, or to deposit iron about the cells.

pe species: *Gaffkya tetragena* (Gaffky) isan.

TES: This genus contains only two es, *G. tetragena* and *G. homari*.

orphology as above. In laboratory cul- the organisms occur as pairs and ir- ar clusters. Individual cells are usually o 1.0 μ in diameter. Nonmotile.

owth occurs on meat infusion agar; ies are circular, smooth, glistening and e, grayish white, and 1 to 2 mm. in eter. The two species require growth rs (biotin, calcium pantothenate, thi- , and nicotinic acid) and some amino for growth.

owth in liquid media is usually in the of a sediment.

itmus milk is acidified.

elatin is not hydrolyzed.

cid but no gas is produced from glucose lactose and from some other carbo- ates.

erobic. Optimal temperature, 37° C.

ffkya tetragena, which gives a viscid e growth on potato, has been demonstrated in sputum and isolated from the skin and air.

Gaffkya homari, which does not grow on potato, causes a fatal disease of lobsters.

Sarcina (Sar.ci′ na) Goodsir, 1842. *M p. 467*. (Edinburgh Med. and Surg. J., *57*, 1842, 430.)

DIFFERENTIATING CHARACTERS: Free-living, spherical, nonphotosynthetic, unicellular organisms which divide in three perpendicular planes to produce cubical packets of eight cells each.

They do not deposit sulfur internally (*cf. Thiosarcina*) or deposit iron on the surface of the cells.

Type species: *Sarcina ventriculi* Goodsir.

NOTES: The genus is best treated in two parts.

Part 1, consisting of *S. ventriculi*, *S. maxima*, *S. methanica*, and *S. barkeri*, covers a group of *anaerobic* species in which the diameter of individual cells is 2.0 μ or more. *S. ventriculi* and *S. maxima* are both 3.5 to 4.5 μ in diameter.

S. ventriculi and *S. maxima* will grow only in sugar-containing peptone media. Both produce acid and gas from glucose, lactose, maltose, fructose, galactose, and sucrose. The gas is CO_2. Other byproducts include ethyl alcohol (*S. ventriculi*) and acetic and butyric acid (*S. maxima*).

S. methanica has been cultivated only in a medium containing calcium acetate or calcium butyrate which it converts to CO_2, *methane*, and calcium carbonate.

S. barkeri can grow autotrophically on CO_2 or CO and heterotrophically on sodium acetate or methanol from which it produces *methane* and CO_2.

Part 2 comprises the remaining six recognized species, all of which are *aerobic*, grow well on meat infusion agar (except *S. littoralis* which is halophilic), producing off-white or distinctly yellow, orange, or coral red colonies. Because of the structural units in the colonies, they usually have a matt surface.

Very little information of value is provided for the differentiation of these species.

They occur in air and water and have an optimal temperature between 20° and 30° C.

Unusual among this group is *Sarcina ureae* which is motile with a single polar flagellum and produces spherical endospores. It is active in the deamination of urea.

Methanococcus (Me.tha.no.coc′ cus) Kluyver and van Niel, emend. Barker, 1936. *M p. 473.* (Kluyver and van Niel, Zentr. Bakteriol. Parasitenk., Abt. II, *94*, 1936, 400; Barker, Arch. Mikrobiol., *7*, 1936, 430.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonphotosynthetic, nonsporing, spherical cells which may exceed 2 μ in diameter; pleomorphic; arranged singly and in masses; Gram-positive; *anaerobic*; ferment certain organic compounds with the production of methane and carbon dioxide.

They do not deposit sulfur internally or iron externally.

Type species: *Methanococcus mazei*, Barker.

NOTES: There are only two species, *M. mazei* and *M. vannielii*. In the former the cells are small and may be aggregated in cysts. In the latter they vary in diameter from 0.5 to 4.0 μ. *M. vannielii* is motile.

M. mazei attacks acetate but not ethanol or butanol. It does not use organic nitrogen compounds.

M. vannielii attacks formate but not acetate, propionate, butyrate, succinate, ethanol, or methanol.

Both species occur in mud and have an optimal temperature between 20° and 37° C.

Peptococcus (Pep.to.coc′ cus) Kluyver and van Niel, 1936. *M p. 474.* (Zentr. Bakteriol. Parasitenk., Abt. II, *94*, 1936, 400.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, spherical organisms less than 2.0 μ in diameter (except *P. glycinophilus*); arranged singly, in pairs, and in irregular clusters; nonmotile; Gram-positive; *anaerobic*; het trophic; do not deposit iron.

Type species: *Peptococcus niger* (H Kluyver and van Niel.

NOTES: Eleven species are recorded. T are differentiated on cultural and bioche cal properties only.

The organisms grow on meat infus agar. Three species may require add glycine. In deep agar cultures 5/11 spec produce gas from the peptone and caus splitting of the agar. One species (*P. nig* produces a water-soluble black pigm which should not be confused with bla ening which occasionally takes place wh H_2S is produced in iron- or lead-contain media.

P. glycinophilus has cells ranging in ameter from 0.7 to 2.5 μ. It is one of species requiring glycine for growth a decomposes it to CO_2, NH_3, and ace acid.

Good growth occurs on blood agar wi out any evidence of hemolysis.

Broth becomes uniformly turbid with g production occurring with 5/11 speci Gas results from the decomposition amino acids with the liberation of CO H_2, and NH_3.

Gelatin is liquefied by 3/11 species.

Litmus milk is unchanged (3/11) rendered slightly acid (1/11) or the litn is reduced without acid production (6/1

Acid is produced from a limited ra of carbohydrates, but lactose is fermen by only one species (*P. grigoroffi*).

Seven species are recorded as catal positive.

The optimal temperature is 37° C.

The species have been isolated pri pally from infections of the natural bo cavities of man, particularly from the mentary, vaginal, and respiratory tra Some of these may be secondary invad

Neisseria (Neis.se′ ri.a) Trevisan, 18 *M p. 480.* (Atti accad. Fisio-Medico-S tistica in Milano, Ser. 4, *3*, 1885, 105.)

DIFFERENTIATING CHARACTERS: Free ing, unicellular, colorless, kidney-shaped

erical organisms occurring basically
with the flat sides adjacent; animal
s; Gram-negative; *aerobic*; hetero-
iron is not deposited by the cells.
species: *Neisseria gonorrhoeae* Tre-

s: The genus has 10 species, all of
are parasites of man or animals.
w exceptions they occur in the res-
tract. Nonmotile.
es differentiation is based on cul-
d biochemical characters only. Pig-
ion and production of acid from
, maltose, fructose, and sucrose are
in points of difference.
also notes on the genera, *Mima*,
, and *Colloides*.

onella (Veil.lo.nel′ la) Prévot,
M p. 485. (Ann. Sci. nat., Bot. 10s.,
3, 118.)
ERENTIATING CHARACTERS: Free-liv-
icellular, colorless, spherical organ-
.3 to 2.0 μ in diameter; arranged in
r in clusters; Gram-negative; do not
e methane from acetate; *anaerobic*;
rophic; do not deposit oxides of iron.
e species: *Veillonella parvula* (Veil-
d Zuber) Prévot.
s: Six species are described. All are
tile. It should be noted that the di-
of cells of individual species range
.3 to 2.0 μ and not 0.3 to 0.4 μ as
ed in the generic description in the
l.
organisms grow well in meat extract
with three of the six species produc-
s from the proteins. Blood is he-
d by only 1/6 species.
ose is fermented with the production
and gas by the type species only.
th cultures of 3/6 species are malo-
.
erobic, obligatory.
mal temperature, 37° C.
nd in natural cavities in the human
Their numbers appear to increase in
diseases of these cavities, but they
ot be associated therewith.

ibacterium (Brev.i.bac.te′ ri.um) Breed, 1953. *M p. 490*. (Riassunti delle Comunicazioni, 6th Cong. intern. microbiol., Rome, *1*, 1953, 13.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, nonsporing, unbranched, rod-shaped organisms less than 2 μ wide; occur singly or in pairs and short chains; nonmotile or motile with flagella; Gram-positive; nonacid-fast; heterotrophic; acid with no gas *or* no reaction in glucose; no acid from lactose.

They *do not or are not known to*: produce propionic acid from lactic acid or alcohol from glucose or deposit iron.

Type species; *Brevibacterium linens* (Weigmann) Breed.

NOTES: Twenty-three species are described. They are separated in the *Manual* on color, motility, action on litmus milk, reduction of nitrate, liquefaction of gelatin, production of acid from maltose, and salt requirements.

It is not possible to define this genus clearly. It was introduced primarily to provide a grouping (taxon) into which an as semblage of Gram-positive, nonsporing nonacid-fast rods, having no clear-cut affinities with other genera of Gram-positive rods, could be placed. Only a general description of the genus can be given.

Unicellular, rod-shaped, unbranched organisms, averaging 0.5 to 1.0 μ by 2 to 5 μ. Gram-positive, nonacid-fast. Of the listed species 17/23 are nonmotile. Of the remaining six, four are listed as peritrichously flagellated, and two as polarly flagellated. The majority are arranged singly and at random. Pairs and short chains do occur. Nonsporing.

The organisms grow on meat infusion agar, four with a sea water base. Colonies range from 1 to 5 mm. in diameter. The type of colony varies with the species. They are either nonpigmented or are various shades of red or yellow.

Gelatin is liquefied by 17/23 species.

Litmus milk is rarely acidified. In most cases litmus is reduced and peptonization occurs.

Indole negative (21/23).

Nitrite is produced from nitrate by 6/19 species.

Acid but no gas is produced from glucose by 14/23 species. Lactose is not fermented (only one species is listed as variable in this respect). Acid is produced from maltose by 8/16 species.

Optimal temperature between 20° and 30° C.

Listed species have been isolated from dairy products, rumen contents, insects, fish, plants, and soil.

Kurthia (Kurth′ i.a) Trevisan, 1885. *M p. 503.* (Atti accad. Fisio-Medico-Statistica in Milano, Ser. 4, *3*, 1885, 92.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, unbranched, rod-shaped organisms less than 2 μ wide and arranged in chains in liquid media; motile with peritrichous flagella; Gram-positive; nonacid-fast; *aerobic*; heterotrophic; no acid from carbohydrates; do not deposit iron; nonpathogenic to plants and animals.

Type species: *Kurthia zopfii* (Kurth) Trevisan.

NOTES: Three species are described. They are rods 0.8 by 1 to 8 μ and are separated in the *Manual* on the basis of gelatin liquefaction and hydrogen sulfide production.

Grow well on meat infusion agar, producing circular or irregular colonies; not pigmented.

Growth in broth is turbid with a flocculent sediment. Broth cultures have a very unpleasant odor attributable to liberation of ammonia.

Gelatin is hydrolyzed by 1/3 species.

Litmus milk is unchanged (2/3) or made alkaline and peptonized (1/3).

No acid or gas is produced from any carbohydrates.

Indole-negative.

Nitrites are not produced from nitrates.

Optimal temperature, 25° to 30° C.

Isolated from hen manure, feces, and putrefying organic matter.

Diplococcus (Dip.lo.coc′ cus) Weichselbaum, 1886. *M p. 507.* (Weiner me
Jahrb., *82*, 1886, 483.)

DIFFERENTIATING CHARACTERS: Free-l
ing, unicellular, colorless, spherical to l
ceolate organisms occurring in pairs, p
ticularly in animal tissues where they
capsulated; chains are common in cult
media; soluble in bile; parasitic; found
the respiratory tract of man. Lactic a
is produced from glucose in small amou
and is dextrorotatory; Gram-positive; *a*
obic; heterotrophic; iron is not deposite

Type species: *Diplococcus pneumoni*
Weichselbaum.

NOTES: There is only the one species.
is nonmotile. The organisms are found
sputum in cases of pneumococcal pne
monia. In sputum they occur in pairs in
common capsule, the organism being la
ceolate and arranged end to end with f
sides adjacent. In liquids the capsules a
frequently lost, and the organisms assu
a more spherical form. (It is possible th
the lanceolate shape is the result of he
fixation before staining (author's note)
Serological typing of the species is based
differences in the capsular polysacchari

The organism grows on meat infusi
agar producing colonies which are 0.5 t
mm. in diameter circular, entire, high co
vex, smooth, and transparent.

On blood agar a greening of the medi
occurs around the colony because of
change in the blood pigment without eff
on the intact blood cells. This is call
alpha (α) hemolysis.

A uniform turbidity is produced in liq
media.

Gelatin is not liquefied.

Litmus milk is acidified and clotted.

Acid only is produced from glucose, l
tose, sucrose, and inulin.

The organisms are soluble in neutral so
tions of 10 per cent bile. This is possi
attributable to acceleration of an autoly
process to which this organism is prone.

The optimal temperature is 37° C.

Found in various inflammatory con
tions of man.

ptococcus (Strep.to.coc′ cus) Ros-
1884. *M p. 508.* (Mikroorganismen
en Wundinfektionskrankheiten des
hen, 1884, 22.)

ERENTIATING CHARACTERS: Free-liv-
icellular, colorless, spherical to ovoid
sms, less than 2 μ in diameter; ar-
in pairs and chains in liquid media;
luble in bile; produce dextrorotatory
acid, if any, from glucose; do not form
Eldredge tubes when growing in
e-yeast extract-tryptone-phosphate
aerobic; heterotrophic; iron is not
ted on the cells.

es species: *Streptococcus pyogenes*
bach.

ES: Nineteen species are described.
occi rarely exceed 1.0 μ in diameter.
formation results from division in
lane only. In some species cells are
with the long axis at right angles to
rection of the chain or parallel to it.
latter, particularly during division,
esemble rod-shaped organisms.

nsient capsule formation occurs in
fluids with some pathogenic species.
species may produce a capsular ma-
of the dextran type similar to *Leu-*
oc but differ from the latter in the
f acid produced.

wth of most species will occur on
infusion agar but is greatly improved
ood or carbohydrates. Colonies are
more than 1 mm. in diameter and are
ar and entire. The surface may vary
mucoid to smooth to rough.

blood agar colonies are often sur-
ed by a zone in which the blood pig-
have undergone some form of alter-
These alterations are classed as
vs.

(β) *hemolysis:* a water-clear zone
d the colony from which the blood
ent and cells have completely disap-
d.

(α) *hemolysis:* a greenish zone around
olonies in which the red blood cells re-
intact, but there is an alteration in
ematin pigments.

Gamma (γ) *hemolysis:* no change at all.

Gelatin is not hydrolyzed except by two varieties of *Streptococcus faecalis* (vars. *liquefaciens* and *zymogenes*).

Litmus milk is acidified by all species except *Strep. equi*, *Strep. acidominimus*, and *Strep. equinus*. With other species simple acidification may occur without clotting or reduction of litmus. In still others clotting occurs. Litmus may also be reduced either before or after clotting, depending on the species.

Acid but no gas is produced from carbohydrates. Lactose is fermented by all species except *Strep. equi* and *Strep. equinus*. The acid produced is dextrorotatory.

Differentiation of species is based on numerous characteristics including hemolysis of blood, decarboxylation of tyrosine, salt and dye tolerances, and growth at limiting pH values.

Serological methods have a special application in the differentiation of the beta hemolytic streptococci.

Aerobic to microaerophilic.

Catalase-negative.

Devoid of cytochrome pigments.

Optimal temperature, 37° C.

Species which are pathogenic to man and animals occur mainly on those hosts. They are responsible for such infections as sore throats and scarlet fever in man, mastitis in cattle, and strangles in horses. The fecal streptococci are found in the alimentary canal of both man and animals. The "lactic" streptococci, which are responsible for the souring of milk and which are used commercially in cheese manufacture, are widespread in milk and milk products.

Pediococcus (Pe.di.o.coc′ cus) Balcke, 1884, emend. Mees, 1934. *M p. 529.* (Balcke, Wochnschr. Brau., *1*, 1884, 257; Mees, Ondersoekingen over de Biersarcina. Thesis, Delft, 1934, 92.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonsporing, nonphotosynthetic, spherical organisms, less than 2 μ in diameter; occur singly, in pairs, and in tetrads with the latter predominating in acid media; Gram-positive; *aerobic*; heterotrophic; produce at least 0.5 to 0.7 per cent of optically inactive lactic acid in yeast extract-glucose-tryptone-phosphate broth, lowering the pH to below 4.0.

Type species: *Pediococcus cerevisiae* Balcke.

NOTES: There are only two species, *P. cerevisiae* and *P. acidilactici*. They occur singly, in pairs and in tetrads, the latter predominating in acid media. Gram-positive. Nonmotile.

They grow on peptone media but growth is improved by the addition of malt. Colonies are small and whitish. Growth factors and amino acids are required.

Gelatin is not liquefied.

Acid and a trace of gas are produced from a wide range of carbohydrates, the pH being lowered usually to 4.0. The principal acid produced is lactic, although a trace of acetic acid may be formed. The lactic acid produced is *inactive*.

Urease-negative.

Nitrites are not produced from nitrates.

Catalase-negative.

Aerobic to microaerophilic.

Optimal temperature, 25° to 32° C. (*P. cerevisiae*) and 41° (*P. acidilactici*).

Found in malt and beer.

Leuconostoc (Leu.co.nos′ toc) van Tieghem, emend. Hucker and Pederson, 1930. *M p. 531*. (van Tieghem, Ann. sci. nat., *6*, Sér. 7, 1878, 170; Hucker and Pederson, N. Y. State Agr. Expt. Sta. Bull., *167*, 1930, 66.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, spherical organisms, less than 2 μ in diameter; arranged in chains; produce gas in Eldredge tubes when growing in yeast extract-glucose-tryptone-phosphate broth. The lactic acid produced is *levorotatory*; frequently produce a copious slime when grown in sucrose broth; Gram-positive; *aerobic*; heterotrophic; iron is not deposited.

Type species: *Leuconostoc mesenteroides* (Cienowski) van Tieghem.

NOTES: Three species differentiated on the fermentation of sucrose and pento are described. Nonmotile. When grow sucrose media the cells of two species *mesenteroides* and *L. dextranicum*) for capsular material which is a dextran.

The organisms grow on meat infus agar but are favored by the addition sugars and yeast extract or vegetable tract. Colonies are small and grayish wh

Gelatin is not liquefied.

Litmus milk is rendered acid and coa lated.

Indole is not produced.

H_2S is not produced.

Nitrites are not produced from nitra

Acid is produced by all species from g cose, lactose, fructose, and galactose. Ot sugars may be attacked.

Lactic acid, acetic acid, and ethyl alco are produced and CO_2 is liberated. All s cies produce a *levorotatory* lactic acid. So dextrorotatory acid may also be produce The amount of CO_2 produced may not sufficient to be detected with Durha tubes. Its evolution is usually demonstrat with Eldredge tubes.

Optimal temperature, 20° to 28° C.

Usually found producing slime in sucro solutions or in milk products.

Peptostreptococcus (Pep.to.strep.t coc′ cus) Kluyver and van Niel, 1936. *M* *533*. (Zentr. Bakteriol. Parasitenk., A II, *94*, 1936, 391, 395, and 401.)

DIFFERENTIATING CHARACTERS: Free-l ing, unicellular, colorless, spherical org isms, less than 2 μ in diameter; arranged pairs and chains only; Gram-positive; *A* *erobic*; heterotrophic; do *not* deposit iron ferment acetate with the production methane.

Type species: *Peptostreptococcus a* *erobius* (Krönig emend. Natvig) Kluy and van Niel.

NOTES: Thirteen species are describ They are differentiated on morpholog and biochemical characteristics. Arrang in pairs or short to long chains and o rarely in clusters (*P. evolutus*). Nonmot Gram-positive.

ve of the 13 species will grow on
fusion agar without special enrich-
ut media containing blood and car-
ates are preferred. Veillon's semi-
ar is used extensively. Colonies of
erent species vary in size from the
inute to 1 to 2 mm. in diameter.

olysis of blood agar is either absent
f the green α type.

ies give a turbid or flocculent growth
tone water. Three species (*P. ana-*
, *P. foetidus*, and *P. productus*) pro-
as from the peptone.

tin is liquefied by only one species
lutus).

is rarely produced from peptone but
ently produced in the decomposition
animal fluids, *e.g.*, blood or serum.

one species (*P. magnus*) fails to
e acid from carbohydrates. Five spe-
o produce gas. Three of these pro-
as from peptone alone.

ng the byproducts of fermentation
tic, formic, valerianic, butyric, pro-
, and lactic acids and CO_2, H_2, and
The fetid odor of some cultures is
the organic acids.

tose is fermented and litmus milk is
ed and curdled by 7/13 species.

species are, on primary isolation,
anaerobic. Three (*P. evolutus*, *P.*
eumoniae, *P. morbillorum*) become
lerant on subculture.

optimal temperature is 37° C.

ies in the genus have been isolated
from putrefactive lesions in the na-
cavities of the human and animal
particularly from the female genital
, gangrenous conditions of various
, and inflammations of the alimen-
anal.

tobacillus (Lac.to.ba.cil′lus) Bei-
k, 1901. *M p. 542.* (Arch. néerl. sci.,
em. Sér. 2, 7, 1901, 212.)

ERENTIATING CHARACTERS: Free-liv-
nicellular, nonphotosynthetic, non-
g, rod-shaped organisms, less than 2
e, which occasionally show bifurca-
and rudimentary branching; occur singly and in chains; nonmotile; Gram-positive; nonacid-fast; *aerobic* or *anaerobic*; heterotrophic; grow poorly on meat infusion agar but well on media containing carbohydrates and yeast extract; colonies are usually 1 mm. or less in diameter and colorless; grow in glucose broth, producing a final pH below 6.0; glucose converted quantitatively by homofermentative species to lactic acid, and by heterofermentative species to lactic acid, acetic acid, alcohol, and carbon dioxide with visible gas produced only by the latter. Acid produced from lactose by all species except *L. delbrueckii* and *L. brevis*; lactic acid not metabolized; not known to survive heating to 72° C. for 15 minutes.

Type species: *Lactobacillus caucasicus* Beijerinck.

NOTES: Fifteen species are described and they are separated in the *Manual* on the nature of byproducts of fermentation, fermentation of lactose, temperature of growth, and optical rotation of acid produced.

The morphology of the organisms varies with the species and conditions of cultivation. Some species, principally those producing only lactic acid from glucose (homofermenters) retain the simple rod-shape throughout their growth. Many of the other species produce uniform rods in the early stages of development but become extremely pleomorphic while the colonies are still microscopic in size. Bifurcations are common with *L. bifidus*.

Growth on meat infusion agar without added carbohydrate is very poor or absent. Optimal growth occurs on yeast or plant extract media containing glucose. Colonies vary in size from less than 1 mm. with most species to 2 to 3 mm. (*L. bifidus*). Pigmentation is rare. Occasional yellow, orange, or rust red colonies are produced.

Gelatin is not liquefied.

Litmus milk is acidified and clotted by 7/15 species, principally the homofermentative types having an optimal temperature of 37° C. or above.

Acid is produced from glucose and maltose by all species. Lactose is fermented by all but *L. delbrueckii*, *L. leichmannii*, and some strains of *L. brevis*. Fructose and galactose are fermented by all species except *L. caucasicus*.

Eleven of the 15 species are homofermentative, producing lactic acid alone or with only traces of volatile acids. The optical rotation of the acid produced varies with the species.

Four of the 15 species are heterofermentative, producing, in addition to lactic acid, appreciable quantities of volatile substances (acetic acid, ethyl alcohol) and CO_2. Copious gas production is noted only with *L. brevis*. The optical rotation of lactic acid produced varies with the species.

Lactate is *not* metabolized.

Catalase-negative.

The optimal temperature for growth varies with the species. All the heterofermentative species grow at 28° to 32° C. as do 3/11 homofermentative species. The others grow best at 37° to 45°. The latter species are important in the industrial production of lactic acid.

Frequently found in fermenting plant juices, nearly always in association with yeasts since both are aciduric. Always found in mature cheese of the cheddar type, in the feces of milk-fed infants, and in the vaginal canal.

Eubacterium* (Eu.bac.te′ri.um) Prévot, 1938. *M p. 552.* (Ann. inst. Pasteur, *60*, 1938, 294.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, unbranched, nonsporing, rod-shaped organisms, less than 2 μ wide; arranged singly, in pairs, in clusters, and occasionally in chains; nonmotile; Gram-positive; nonacid-fast; *anaerobic*; heterotrophic; grow in peptone water, often producing gas in the absence of carbohyhydrates (13/20); produce acid (and possibly gas) from carbohydrates (17/20). acid byproducts vary with the species include propionic, butyric, lactic, ac and formic acids.

Type species: *Eubacterium foed* (Klein) Prévot.

NOTES: The differentiating charact given above are not very satisfactory. genus differs mainly from some species *Propionibacterium* and *Butyribacterium* its apparent ease of growth in pepto media and from *Ramibacterium* in the rangement of cells. The differentiation some species from *Catenabacterium* is clear. In the *Manual* the keys given on 542 separate *Eubacterium* on lack of ch formation, yet in keys to *Eubacterium* p. 552 species are specifically separated chain formation.

Twenty species are described. They separated in the *Manual* on production gas in media, odor, liquefaction of gelat action on milk, blood hemolysis, arrang ment of cells, production of ethanol, duction of nitrates, reduction of neut red and phenosafranine, and on path genicity.

All species grow in peptone water; 13/ species produce *gas* from peptone, a many of these have an offensive odor, a tributable to the liberation of volat amines and ammonia.

Gelatin is liquefied by only 4/20 speci

Coagulated serum is liquefied by o one. Acid is produced from a variety carbohydrates by 15/20 species. The coi cident gas production by some species m be from peptone. *Propionic*, *butyric*, (*Propionibacterium* and *Butyribacteriu* lactic, acetic, formic, and valerianic aci are produced. A few also produce ald hydes, ketones, and acetylmethylcarbin

Indole is not produced.

All species are *anaerobic* and have be isolated from a variety of gangrenous a suppurative infections in various parts the body; also from feces of man and a mals and decomposing meat.

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* The name *Eubacterium* as used by Prévot is a later homonym of *Eubacterium* Janke and hence is illegitimate (Buchanan, private communication).

al species are pathogenic to labora- mals.

nabacterium (Ca.te.na.bac.te′ ri. évot, 1938. *M p. 560.* (Ann. inst. , *60*, 1938, 294.)

RENTIATING CHARACTERS: Free-liv- nicellular, colorless, nonsporing, or curved rod-shaped organisms, n 2 μ wide; occur as S-shaped, fila- s, pleomorphic forms or as regular ped cells in long chains; nonmotile; ositive, nonacid-fast; *anaerobic*; rophic; may produce gas from pep- /7); ferment glucose with the pro- of *propionic*, lactic, *butyric*, acetic, mic acids, volatile amines, alcohol, les, and acetoin.

species: *Catenabacterium helmin-* (Lewkowicz) Prévot.

s: They are separated in the *Manual* production, liquefaction of gelatin, tion of milk, growth requirements, ltural characteristics.

genus has seven species. With one on they grow in peptone media with- ded carbohydrates or serum. The pecies producing gas from peptone pronounced fetid odor. Byproducts wth in glucose-peptone water by pecies include propionic acid (2/3), cid (3/3), acetic acid (1/3), butyric /3), volatile amines (3/3), formic /3), alcohol (3/3), aldehydes (1/3), etylmethylcarbinol (3/3).

ic and butyric are produced by one nongas-forming species. The type of uct is not specified for the others. distinction between the genus and of *Propionibacterium* and *Butyri- um* is not clear. Further information species is required before a decision reached.

ies have been isolated from cases of licitis, gangrene of the lung, uterine ons, pleurisy, dental caries, the nor- outh, and from pond mud.

ibacterium (Ra.mi.bac.te′ ri.um) , 1938. *M p. 563.* (Ann. inst. Pas- *0*, 1938, 294.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, straight or curved rods, less than 2 μ wide and arranged in Y- or V-shaped formations *suggestive* of branching; nonmotile; Gram-positive; nonacid-fast; *anaerobic*; heterotrophic; growth in peptone water, in the absence of carbohydrates, with the production of gas. Acids, principally lactic, acetic, butyric, propionic, formic, and valerianic, are produced from carbohydrates.

Type species: *Ramibacterium ramosum* (Veillon and Zuber) Prévot.

NOTES: Six species are described. They are separated in the *Manual* on the production of indole, coagulation of milk, reduction of neutral red, and production of organic acid.

The above differentiating characteristics are not very satisfactory. The principal difference from *Catenabacterium* and *Eubacterium* is the arrangement of the cells. The species are clearly differentiated from *Propionibacterium* and *Butyribacterium* by the production of gas from and growth in peptone.

Gelatin is not liquefied.

Litmus milk is acidified and clotted by 3/6 species.

Ammonia is produced from peptone.

Alcohols, ketones, volatile amines, and acetoin are also produced.

Species have been isolated from cases of dental infection, pleurisy, and appendicitis and from feces. Some are pathogenic to laboratory animals.

Cillobacterium (Cil.lo.bac.te′ ri.um) Prévot, 1938. *M p. 566.* (Ann. inst. Pasteur, *60*, 1938, 294.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, unbranched, straight or curved rod-shaped organisms; pleomorphic; *motile* with peritrichous flagella; Gram-positive; nonacid-fast; *anaerobic*; heterotrophic; grow in peptone water with the production of gas; carbohydrates fermented by 4/5 species with the production of lactic, acetic, butyric, valerianic, and formic acids.

Type species: *Cillobacterium moniliforme* Prévot.

NOTES: Six species are described. They are separated in the *Manual* on gelatin liquefaction, coagulation of milk, fermentation of glucose and cultural characteristics.

Biochemical tests have not been uniformly applied but the following observations have been made.

Indole is produced by 4/4 species.

Nitrites are produced from nitrates by 3/3.

Neutral red is reduced by 4/5.

Gelatin is liquefied by 5/6 species.

Litmus milk is not rendered acid or alkaline, although gas may be produced.

Optimal temperature, 37° C.

The organisms have been found in lesions of warm-blooded animals and in the intestinal tract. Most species are pathogenic to the guinea pig.

Propionibacterium (Pro.pi.on.i.bac.ter′i.um) Orla-Jensen, 1909. *M p. 569.* (Zentr. Bakteriol. Parasitenk., Abt. II, *22*, 1909, 337.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, nonsporing organisms, less than 2 μ wide; pleomorphic, occurring as chains of cocci in acid media under anaerobic conditions or as rods with occasional rudimentary branching under aerobic conditions; nonmotile; Gram-positive; nonacid-fast; heterotrophic; poor growth in media without added carbohydrate; produce propionic acid, acetic acid, and carbon dioxide from glucose *and from lactic acid*; catalase-positive.

Type species: *Propionibacterium freudenreichii* van Niel.

NOTES: There are 11 recognized species. All of these, when grown aerobically in yeast extract media containing carbohydrates are very pleomorphic, club-shaped, and branched rods. Nonmotile and nonsporing.

Anaerobically the morphology of cells is more uniform but the shape of cells frequently differs in neutral media from t in acid media; the former are spherical resemble streptococci, but the latter usually rod-shaped.

Growth on peptone media without bohydrates is very poor or absent. All cies examined require pantothenic acid biotin as growth factors. Two require aminobenzoic acid, and all are *stimula by* but do not require thiamin.

Growth in stab cultures is usually go Color varies with the species. *P. rubr* and *P. thoenii* are brownish red. Ot species vary from cream to yellow orange, the darker color appearing in more aerobic growth near the top of stab.

Colonies are usually moist and butyro Those of *P. peterssonii* are dry.

In a yeast-peptone medium *all* spec ferment *lactic acid*, pyruvic acid, di droxyacetone, and glycerol to *propio* acid, acetic acid, and CO_2. The amount CO_2 formed may be too small to be tected by the Durham tube method.

With the exception of *P. freudenreic* and *P. shermanii all* species produce pr pionic acid from glucose, lactose, maltos fructose, mannose, galactose, and sucro but *not* from inulin or dulcitol.

P. freudenreichii does not ferment la tose, maltose, or sucrose, and *P. sherma* fails to ferment maltose and sucrose.

Differentiation of species is based their action on other carbohydrates.

P. arabinosum and *P. pentosaceum* notable for their action on pentoses.

Litmus milk is slowly acidified clotted by all but *P. arabinosum.*

Indole is not produced.

Gelatin is not liquefied.

Nitrates are not reduced to nitrites any species except *P. pentosaceum* wh reduces through nitrite to nitrogen.

Catalase-positive (*P. arabinosum* weak).

Optimal temperature, 30° C.

Found in dairy products, particularl

ole" cheese of the Gruyère and
ypes.

ribacterium (Bu.ty.ri.bac.te′
Barker and Haas, 1944. *M p. 577.*
teriol., *47*, 1944, 301.)

ERENTIATING CHARACTERS: Free-liv-
nicellular, colorless, unbranched,
ring, rod-shaped organisms, less
μ wide; arranged singly and in short
nonmotile; Gram-positive; nonacid-
aerobic; heterotrophic; acid is pro-
from glucose and maltose but not
ctose or fructose; *butyric acid*, acetic
nd carbon dioxide produced from
, maltose, *and also from lactic acid.*

species: *Butyribacterium rettgeri*
and Haas.

s: There is only one species listed.
t or slightly curved, unicellular, rod-
organisms, 0.7 by 2.3 μ.

rowth occurs on meat infusion agar.
es, 2 mm. in diameter in 4 days at
are produced on glucose-cysteine
tryptone yeast-extract lactate agar.
es are circular, translucent, grayish
convex to umbonate, glistening, and
. Edges are entire or slightly irregu-

ic acid, butyric acid, and CO_2 are
ed from glucose and maltose. The
y not be sufficient to be evolved.

se, arabinose, lactose, sucrose, tre-
rhamnose, mannitol, sorbitol, dulci-
l glycerol are not fermented.

te is readily fermented and converted
ic acid without visible gas produc-

le-negative.

negative.

lase-negative.

mal temperature, 37°C.

ted from the intestine of the white

obacterium (Zy.mo.bac.te′ ri.um)
man and Barker, 1954. *M p. 577.*
teriol., *68*, 1954, 400.)

ERENTIATING CHARACTERS: Free-liv-
icellular, colorless, unbranched, non-
, spindle-shaped rods, less than 2 μ
wide; occur as long intertwined chains in liquid media; nonmotile; Gram-positive; nonacid-fast; *aerobic* to *anaerobic*; heterotrophic; acid and gas produced from glucose in glucose-peptone water; glucose fermented in a yeast-like manner with the production of ethyl alcohol and small amounts of acetic acid and carbon dioxide.

Type species: *Zymobacterium oroticum* Wachsman and Barker.

NOTES: There is only one listed species. Rod-shaped organisms, 0.3 to 0.6 μ by 1.2 to 2.0 μ with tapering ends, usually occurring in long intertwined chains.

Growth on meat infusion media is not stated.

Small, round, convex colonies are produced on tryptone yeast-extract glucose agar.

No growth in milk.

Indole is not produced.

Acid and gas is produced from glucose, fructose, sucrose, lactose, maltose, arabinose, galactose, and mannitol. Lactic acid, glutamic acid, and glycerol are not utilized.

Glucose fermentation yields predominantly ethyl alcohol (1.3 moles per mole of glucose) and CO_2 and possibly lactic and formic acid. The fermentation is slow.

Orotic acid is fermented with the production of ammonia.

Starch is not hydrolyzed.

Nitrites are not produced from nitrates.

Catalase-negative.

Anaerobic to microaerophilic.

Isolated from bay mud.

Corynebacterium (Co.ry.ne.bac.te′ ri.um) Lehmann and Neumann. *M p. 579.* (Bakteriol. Diag., 1 Aufl., *2*, 1896, 390.)

DIFFERENTIATING CHARACTERS: Free-living, nonphotosynthetic, nonsporing, rod-shaped organisms, less than 2 μ wide, which appear in smears in palisade or Chinese letter forms; rarely show rudimentary branching; nonmotile (29/33) or motile with polar flagella (4/33); Gram-positive; nonacid-fast; *aerobic* (29/33) or *anaerobic* (4/33); heterotrophic; acid produced from glucose (27/33) and lactose (15/33); gas also produced by 2/33. Butyric acid is not recorded among the byproducts and propionic acid, recorded for only one species, is not produced from lactic acid.

Type species: *Corynebacterium diphtheriae* (Flügge) Lehmann and Neumann.

NOTES: The distinction between this genus and the genus *Brevibacterium* is not very clear. The organisms in the genus *Brevibacterium* are considered to be uniform, nonpleomorphic rods arranged singly and at random. Most, but not all, species of the genus *Corynebacterium* are very pleomorphic cells with clubbed ends, curved axes, and irregular staining. The latter character has been shown in some species only to be due to a multicellular nature of each organism, although this is *not apparent without special stains applied to demonstrate cell walls.* This may be the most useful criterion for separation from *Brevibacterium.*

The cells of many species of the genus *Corynebacterium*, when examined in stained smears from agar plate cultures, appear to be arranged in palisades or Chinese letter forms, a characteristic attributed by most authors to a peculiar mode of division which results in the temporary adherence of cells after division. The fact that such cells, once dispersed in a fluid so that they occur singly when smears are examined immediately, are found to have regrouped in palisades on standing suggests an entirely different explanation for this phenomenon based on cell surface characteristics (author's note).

The organisms will grow on meat infusion agar. Colonies vary with the species but usually exceed 1 mm. in diameter, are circular entire and butyrous. They are either grayish white or pigmented cream, yellow, or red. Blue granules are produced in yellow colonies of *C. insidiosum.*

Gelatin is liquefied by 10/33 species.

Litmus milk is rarely acidified even by species which ferment lactose in peptone or serum bases. It is sometimes turned al line and only occasionally peptonized.

Acid but no gas is produced from gluc by 27/33 species. Two anaerobic spec (*C. avidum* and *C. diphtheroides*) produ gas.

Several other sugars are fermented different species.

Indole production is recorded for on 3/33 species.

Aerobic except *C. acnes*, *C. parvum*, *granulosum*, *C. avidum*, and *C. diphth roides.*

The optimal temperature varies wi the habitat. Animal parasites and ce mensals have an optimum of 37° C. Thos isolated from plant diseases, plants, so and so on have an optimum closer to 30°.

Approximately half the aerobic speci cause infections in animals. *C. diphtheri* is the cause of diphtheria. The othe mainly cause caseous lesions in variou parts of the body.

The remaining aerobic species are path gens of plants.

Listeria (Lis.te′ ri.a) Pirie, 1940. *M* *597.* (Science, *91*, 1940, 383.)

DIFFERENTIATING CHARACTERS: Free-li ing, unicellular, colorless, nonsporing, u branched, straight, rod-shaped organism motile with peritrichous flagella; Gra positive; nonacid-fast; *aerobic*; hete trophic; produce acid but no gas from g cose, salicin, and esculin; do not depo iron; pathogenic to warm-blooded anima causing monocytosis.

Type species: *Listeria monocytoge* (Murray *et al.*) Pirie.

NOTES: There is only one species. It i rod-shaped organism, 0.4 to 0.5 μ by to 2.0 μ, arranged singly.

The organism grows well on nutr agar, producing circular, smooth, butyro milky colonies, 1 to 2 mm. in diamete

Gelatin is not liquefied.

Litmus milk is acidified and reduced

Indole is not produced.

H_2S is not produced.

Nitrites are not produced from nitra

but no gas is produced from glucose, maltose, sucrose, trehalose, melibirch, rhamnose, glycerol, salicin, and . Mannose, xylose, arabinose, manulcitol, inositol, and inulin are not d.

bic. Optimal temperature, 37° C.

organism is pathogenic to man and s, producing a disease characterized narked increase in the number of uclear leukocytes in the blood.

ipelothrix (E.ry.si.pe′ lo.thrix) ach, 1909. *M p. 599.* (Ztschr. f. *33*, 1909, 367.)

ERENTIATING CHARACTERS: Free-livicellular, colorless, nonsporing, uned, rod-shaped organisms; occur in chains and as long filaments; tile; Gram-positive; nonacid-fast; ; heterotrophic; grow on meat inagar, producing pinpoint transparent s on agar in 24 hours at 37° C. which on further incubation to 1.5 mm.; id produced from glucose and lactose me other carbohydrates but not sculin; final pH in glucose broth .0; H_2S produced; pathogenic, causine erysipelas, human erysipeloid, septicemia, and infections in sheep, and fish.

species: *Erysipelothrix insidiosa* an) Langford and Hansen.

s: There is only one species. The e 0.2 to 0.4 μ by 0.5 to 2.5 μ. Filaare common in rough colonies.

nies on meat infusion agar are barely in 24 hours at 37° C. but enlarge to .5 mm. in 48 to 72 hours. They are rent with a bluish sheen by reflected ircular and entire.

tellurite agar they are an intense

st tube brush-like growth appears in in stab.

us milk is not changed.

le is not produced.

is produced.

but no gas is produced from glucose, galactose, fructose, and lactose. Other sugars may be fermented.

Esculin is not hydrolyzed.

The final pH in yeast-extract trypticase glucose broth is about 6.0.

Nitrites are not produced from nitrate.

Catalase-negative.

Aerobic.

Temperature range 16° to 41° C; optimum, 37°.

Optimal pH, 7.4 to 7.8.

The organism tolerates 7 per cent NaCl, 0.2 per cent phenol, and 0.05 per cent potassium tellurite.

Microbacterium (Mic.ro.bac.te′ ri.um) Orla-Jensen, 1919. *M p. 600.* (The Lactic Acid Bacteria, 1919, 179.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, unbranched, nonsporing, rod-shaped organisms, less than 2 μ wide, arranged in angular fashion similar to *Corynebacterium*; nonmotile;* Gram-positive; nonacid-fast; *aerobic*; heterotrophic; grow well on meat infusion agar producing grayish or yellowish colonies; produce acid but no gas from carbohydrates; glucose, fructose, and mannose fermented; catalase-positive; resist heating to 72° C. for 15 minutes.

Type species: *Microbacterium lacticum* Orla-Jensen.

NOTES: There are only two species, *M. lacticum* and *M. flavum*, listed in the *Manual.* They differ in their action on starch and maltose.

Gelatin is not liquefied.

Litmus milk is slightly acidified or not changed.

Indole is not produced.

Hydrogen sulfide is not produced.

Lactose and maltose are fermented by *M. lacticum* only.

Starch is hydrolyzed by *M. lacticum.*

Nonlipolytic.

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* A third species which is motile with peritrichous flagella has been described by Bolcato (Antonie van Leeuwenhoek. J. Microbiol. Serol., *23*, 1957, 355).

Ammonia is not produced from peptone or arginine.

Found in dairy products.

Cellulomonas (Cel.lu.lo.mo′ nas) Bergey *et al.*, 1923, 1952. *M p. 601.* (Bergey *et al.*, Bergey's Manual of Determinative Bacteriology, 1st ed., 1923; Clark, Intern. Bull. Bacteriol. Nomen. and Taxon., *2*, 1952, 50.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, nonsporing, straight or curved, rod-shaped organisms, less than 2 μ wide; unbranched or showing only rudimentary branching; arranged singly or in palisades; motile with peritrichous flagella or nonmotile; Gram-variable; nonacid-fast; *aerobic*; heterotrophic; produce acid but no gas from glucose and lactose (except *C. acidula*); grow in 0.5 per cent peptone water containing a filter paper strip and completely disintegrate or markedly weaken the paper; produce clearing in a precipitated cellulose agar plate.

They *do not or are not known to:* hydrolyze agar, utilize phenol, oxidize ethyl alcohol quantitatively to acetic acid, produce ethyl alcohol in quantities from glucose, fix gaseous nitrogen, or deposit iron.

Type species: *Cellulomonas biazotea* (Kellerman *et al.*) Bergey *et al.*

NOTES: Pleomorphic, unicellular, rod-shaped organisms, 0.3 to 0.8 μ by 1 to 2 μ, capable of hydrolyzing cellulose when grown in 0.5 per cent peptone.

There are 10 listed species, six of which are motile with peritrichous flagella. The Gram stain is reported to be variable.

The organisms grow well on meat infusion agar. The colonies are translucent to opaque and may be milky white to yellow.

Broth cultures are turbid.

Gelatin is hydrolyzed by all species except *C. acidula*.

Starch is hydrolyzed by all species.

Nitrite is produced from nitrate by 7/10 species.

Acid but no gas is produced from glucose, lactose, maltose, sucrose, and starch by all species except *C. acidula* for which only glucose reaction is recorded.

Cellulose is hydrolyzed when 0.5 per peptone is used as the base.

Optimal temperature, 28° C.

The organisms are found in soil and composing plant tissue.

Arthrobacter (Ar.thro.bac′ ter) Co and Dimmick, 1947. *M p. 605.* (J. Ba teriol., *54*, 1947, 300.)

DIFFERENTIATING CHARACTERS: Free-li ing, unicellular, nonphotosynthetic, no sporing organisms, less than 2 μ wide whi exhibit a definite cycle of development. aged cultures they are coccoid. In so species the cells are very uniform in si and resemble micrococci. In others the coc vary in size, with some cells, called *cystit* several times larger than the majorit When transferred to a fresh medium cocci "germinate" to produce rod-shape cells. Germination may occur at more tha one point which often gives rise to an a pearance of branching. The newly forme rods elongate and divide. At the point division the rods grow out at an angle to t original axis until the total length is a proximately twice the original. This for of growth simulates branching which do *not* occur. Division occurs at the angle an the elongation and division is repeated. older cultures the rods transform into mass of cocci which completes the cyc Gram-variable; *aerobic*; heterotrophic.

Type species: *Arthrobacter globiform* (Conn) Conn and Dimmick.

NOTES: Nonmotile except *A. simplex* *A. citreus.*

The *Manual* (seventh edition) lists species, eight of which grow readily on m infusion agar. Six will also use ammo nitrogen and grow well on soil infus media. Colonies are usually 1 to 2 mm. diameter in 48 hours and are opaque cream to yellowish.

A. terregens will not grow on pept media without the addition of soil extr or an extract of *Arthrobacter pascens.*

When growing on nicotine agar, *Art*

ydans produces a soluble blue pig-

in is hydrolyzed by all species ex-
terregens.
ıs milk is unchanged (2/9) or be-
lkaline and is later peptonized.
h is hydrolyzed by 5/9 species.
only is produced from glucose by
ies. A few other carbohydrates are
ed.
-Proskauer-negative.
e negative (9/9).
es are produced from nitrate by
cies.
ase-positive (except *A. tumescens*).
nal temperature, 20° to 25° C.
d in soil and on plants.
llus (Ba.cil′lus) Cohn, 1872. *M*
(Beitr. Biol. Pflanzen., *1*, Heft 2,
46 and 175.)
RENTIATING CHARACTERS: Free-liv-
icellular, nonphotosynthetic, rod-
organisms, less than 2 μ wide, which
endospores; motile with peritri-
flagella or nonmotile; arranged
or in chains; *aerobic*; heterotrophic;
deposit iron on the cells or fix gase-
ogen.
species: *Bacillus subtilis* Cohn,
Prazmowski.
s: Twenty-five species are described.
tiation is based on morphological
ers and a wide range of physiologi-
acters. As with most genera not all
have been uniformly examined so
oper comparisons cannot be made.
lowing comments are restricted to
haracters which have been uni-
examined.
ellular, rod-shaped organisms which
e elliptical or spherical endospores.
of the 25 listed species the width of
dospore exceeds that of the vegeta-
d and results in a swelling of the rod
central, subterminal, or terminal
n, depending on the position of the
The resulting cells have fusiform,
, or racket-shaped appearances. In
aining 10 species the width of the

endospore does not exceed that of the vegetative cell. It is important to recognize the fact that more than 50 per cent of the species of *Bacillus* have spores which are wider than the rod. Such swollen spores are frequently referred to as *clostridial*, a term which creates confusion with the genus *Clostridium*, a group of *anaerobic* sporing rods in which the swollen spore predominates.

Considered as a genus the vegetative cells of *Bacillus* fall into two main size groups: (*a*) those with cells greater than 0.9 μ in width (usually 1.0 to 1.5 μ) of which there are four listed species, including the anthrax bacillus (*B. anthracis*); and (*b*) those with cells less than 0.9 μ in width (0.3 to 0.9 μ).

Cells may have rounded or square ends and are arranged singly, in pairs, and not infrequently in chains. Only two species (*B. anthracis* and *B. popilliae*) are listed as nonmotile. The others are peritrichously flagellated. A few species are capsulated.

The genus is traditionally regarded as Gram-positive. Two species are listed as Gram-negative and 10 others as Gram-variable.

All species except *B. larvae* grow well on meat infusion agar. Several species have been listed as using ammonia nitrogen but the conditions of the test leave this open to question.

The colony type varies with the species and also markedly within a species on different media under different conditions of growth. Rhizoid, fimbriate, crenate, serate, lobate, undulate, and entire colonies are common. They vary from 1 to several mm. in width. *Bacillus circulans* is most unusual. The entire colony is mobile and migrates over the medium following a watchspring course. *B. alvei* spreads rapidly over the medium in a manner similar to *Proteus*. Most colonies are opaque and whitish. A few are pigmenetd.

Only 3/25 species do *not* liquefy gelatin. The majority also actively hydrolyze casein resulting in peptonization and an alkaline reaction in milk.

The marked liberation of ammonia in protein and peptone media may lead to complete neutralization of acids produced from carbohydrates and so result in a false negative reaction in peptone sugars. For this reason sugar fermentations in the Smith classification of the genus are done concurrently in mineral media with ammonia nitrogen. Since the media are initially neutral, acid production is readily detected.

Starch is hydrolyzed by 14/25 species.

Acid and gas are produced from carbohydrates by *B. polymyxa* and *B. macerans*. All other species produce acid only or no reaction.

All species are *aerobic*.

The optimal temperature varies with the habitat. *B. stearothermophilus* has an optimum between 50° and 65° C. and does not grow at 37°.

Found mainly in soil.

A few species are pathogenic. *B. anthracis* causes anthrax in man and animals. *B. pulvifaciens* and *B. larvae* cause diseases of honey bee larvae, and *B. popilliae* and *B. lentimorbus*, diseases of the larvae of the Japanese beetle.

Clostridium (Clos.tri′di.um) Prazmowski, 1880. *M p. 634*. (Untersuchungen uber die Entwickelungsgeschichte und Fermentwirkung einiger Bacterien-Arten, Inaug. Diss., Leipzig, 1880, 23.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonbranching, nonphotosynthetic, rod-shaped organisms, not more than 2 μ wide; motile with peritrichous flagella or nonmotile; endospores are produced; *anaerobic*;* heterotrophic; iron is not deposited around the cells.

Type species: *Clostridium butyricum* Prazmowski.

NOTES: Ninety-three species are scribed. Included in the genus as an pendix is a description of *Methanobacter omelianski*. This organism oxidizes prim alcohols to acids and secondary alcohol ketones while reducing CO_2 quantitati to methane; glucose is not metabolized.

Differentiation in the genus is based morphological and physiological charac istics. The vegetative rods vary in wi from 0.3 to 2.0 μ and in length from 1.5 12 μ. Straight or slightly curved; occasi ally pleomorphic and twisted or spin shaped. Motile (78/93) with peritrich flagella or nonmotile (13/93). Motilit not recorded for two species. Occur sing in pairs, and frequently in chains, depe ing on the species.

The Gram stain is recorded as negat for 15/93 species. The remainder are Gra positive. Several of the latter are repor as Gram-variable; usually these are Gra positive in very young cultures.

Thirteen species are recorded as gra lose-positive. Granulose is a substa found in cells in a granular form and wh stains blue with iodine.

All species produce endospores, althou in some instances only under critical con tions. Endospores are oval, cylindrical, spherical and occur in a central, su terminal, or terminal position, depend on the species. In only three species is width of the spores less than that of vegetative cell. In the remainder swell of the rods ranges from a minor type which the slight bulge gives the ro spindle shape to pronounced bulging wh results in club-, racquet-, and spo shaped cells, depending on the shape position of the spore and the relative si of the rod and the spore.

There is no record of growth occur in synthetic media with ammonia nitro The majority of species grow on m infusion agar. Some require more com media. Fourteen of the 36 species for wh the reaction on blood is recorded hemolytic. Colonies vary with the spe

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* All species except *Cl. carnis*, *Cl. histolyticum*, *Cl. lacunarum*, *Cl. tertium*, and *Cl. pectinovorum* are strict anaerobes. These five species are regarded as anaerobic but aerotolerant to a certain degree.

minute, colorless colonies; others
and spreading. The conditions of
on in sealed containers normally
a high relative humidity which
spreading types.

pecies produce brightly pigmented
ange colonies, *viz.*, *Cl. venturellii*,
m, *Cl. saturnirubrum*, *Cl. chromo-*
. corallinum, *Cl. aurantibutyricum*,
lsineum. Others produce yellowish
.

is liquefied by 39/87 species. Six
ecorded.

r's inspissated serum is liquefied by
pecies for which the reaction is
.

l meat medium is turned slightly
m its original brown and under-
subsequent blackening or diges-
h a number of species. Gas is
ly produced but is rarely malo-
This reaction is characteristic of
pecies with *strong saccharolytic*
but with only weak or no pro-
activity.

species initially turn the meat
but this is rapidly followed by a
ng of the meat (attributable to re-
etween H_2S produced and Fe from
d pigments) and its subsequent
. Such cultures are exceedingly
us because of the production of
mercaptans, and H_2S. Species
ause this series of changes are
saccharolytic and strongly pro-

species cause no change in the
ce or color of the meat or may
slight blackening without digestion
of production of H_2S from soluble
material or from sulfates.

hydrates are fermented with the
ion of acid and gas by 82/93 spe-
e remainder do not ferment carbo-
. Several of the above species *pro-*
from peptone water alone.

yproducts of sugar fermentations
(*a*) acetic, butyric, formic, pro-
Cl. virens and *Cl. propionicum* (?)),
and lactic acids (*Cl. pectinovorum*); (*b*) isopropyl alcohol (*Cl. aurantibutyricum* and *Cl. pectinovorum*); (*c*) *both* ethyl and butyl alcohols (*Cl. acetobutylicum*, *Cl. kaneboi*, *Cl. butylicum*, *Cl. beijerinckii*, *Cl. iodophilum*, *Cl. toanum*, *Cl. aurantibutylicum*, *Cl. felsineum*, and *Cl. flavum*); (*d*) butyl alcohol (*Cl. muelleri* and *Cl. saturnirubrum*); and (*e*) ethyl alcohol (*Cl. carbonei*, *Cl. spumarum*, *Cl. cellobioparum*, *Cl. dissolvens*, *Cl. thermocellum*, and *Cl. thermocellulaseum*).

Care should be exercised in interpreting sugar fermentation with the genus *Clostridium*. Some species may reduce the acid-base indicator to a colorless substance without producing acid. When bromcresol purple is used such reduced tubes closely resemble tubes in which acid is produced but are usually much lighter in color. Another indicator should be added to check this.

Similarly gas may be produced without acid production.

Litmus milk undergoes numerous changes, depending on the species. Purely saccharolytic species which ferment lactose will produce acid, coagulate the casein, and reduce the litmus. If gas is produced the curd may be shattered (*e.g.*, *Cl. perfringens*).

Strongly proteolytic types will normally cause complete peptonization and leave the milk like a yellow, oily liquid.

Other species may cause no change.

Starch hydrolysis and subsequent fermentation is recorded for 31 species of which 18 are recorded as negtive. Some species react differently on corn and potato starch.

Cellulose hydrolysis and fermentation occur with 10 of the 23 species for which the reaction is recorded. The gas produced frequently has a high content (67 per cent) of methane. (*cf. Methanobacterium omelianski*).

Pectins are hydrolyzed by 10/12 species.

Indole is produced by 11/68 species.

Nitrite is produced from nitrate by 14/63 species.

Atmospheric nitrogen fixation is recorded for 11 species. In most cases the quantity is so small as to be detectable only by isotope procedures. *Cl. butyricum* is the classical nitrogen-fixing anaerobe.

PATHOGENICITY: Twenty-two of the listed species are pathogenic to one or more laboratory animals.

The following species, exclusively soil organisms, have been recorded from cases of gas gangrene in man or animals. They are mainly strongly saccharolytic species: *Cl. multifermentans*, *Cl. gummosum*, *Cl. fallax*, *Cl. septicum*, *Cl. chauvoei*, *Cl. novyi*, *Cl. aerofoetidum*, *Cl. sporogenes* (rarely), *Cl. bifermentans*, *Cl. perfringens*, *Cl. sphenoides*, *Cl. innominatum*, *Cl. paraputrificum*, *Cl. capitovale*.

Cl. botulinum Type A: the cause of botulism, a toxic disease; it is found in soil and produces a powerful exotoxin which is responsible for food-poisoning.

Cl. botulinum Type C: causes limberneck in chickens.

Cl. chauvoei: causes blackleg in cattle.

Cl. haemolyticum: causes icterohemoglobinuria in cattle.

Cl. novyi Type B: causes black disease of sheep.

Cl. tetani: a nonsaccharolytic and weakly proteolytic species; the cause of tetanus.

Cl. felsineum and other pectolytic species are used in the retting of flax.

Cl. acetobutylicum is used for the commercial production of butyl alcohol and acetone.

Optimal temperature varies with the habitat. Some species are thermophilic (*Cl. thermocellum* and *Cl. thermocellulaseum* have an optimal temperature of 55° to 60° C.).

Mycobacterium (My.co.bac.te′ri.um) Lehmann and Neumann, 1896. *M p. 695.* (Bakteriol. Diag., 1 Aufl., *2*, 1896, 108)

DIFFERENTIATING CHARACTERS: Free-living, nonphotosynthetic, rod-shaped organisms, less than 2 μ wide, which may be unbranched or show rudimentary bran ing only; nonmotile; acid-fast; no en spores produced; Gram-positive; het tropic; not known to deposit iron about cells.

Type species: *Mycobacterium tuber losis* (Zopf) Lehmann and Neumann.

NOTES: Fourteen species are listed in *Manual.* The rods are usually irregular shape, the long axis being twisted. casional branches have been repor among the cells of four species (*M. smeg tis*, *M. phlei*, *M. fortuitum*, and *M. tub culosis*). Because of their hydropho nature some species do not stain read with water-soluble dyes.

According to Bisset (J. Gen. Microbi *3*, 1949, 93) these organisms are mu cellular. Only a few cultures have be examined.

The organisms are heterotrophic. S species can be cultivated on media w simple carbon compounds such as citra succinate, and malate with ammonia nit gen.

Parasites of cold-blooded animals a fish are cultured readily on meat infusi agar.

Pathogens of warm-blooded animals quire complex media for good growth a develop slowly even on these. Media co monly employed usually contain glyce and egg.

M. paratuberculosis appears to requ an unspecified component of tubercle other acid-fast bacteria for primary ture.

M. leprae and *M. lepraemurium* have been cultivated on artificial media.

Colonies vary with the species. Some dry, wrinkled, and crumb-like and may pigmented, usually yellow or ora Others are smooth, moist, and butyr The dry colonies are difficult to emulsi

Slight gelatin liquefaction is recorded *M. phlei* only.

Reactions in litmus milk are recorded only six species. One species produces and coagulation (*M. marinum*). The ot

no change (four species) or a
lkalinity (one).
but no gas is produced from glucose
ne other carbohydrates by three of
ed species. Lactose is never fer-
. Some carbohydrates are assimi-
ithout acid production.
e is not produced.
tes are produced from nitrates by
e soil species.
nal temperature lies between 25°
C., depending on the species.
OGENICITY: Diseases caused are
elow.
i and M. smegmatis: saprophytic.
uitum; a soil form which has been
l from infections of the lymph glands
e and abscesses in man.
rinum: causes tuberculosis of fish
gs and also infects mice and pigeons.
nnopheos: a parasite of snakes.
erans: causes ulcers in man and
rats and mice.
berculosis: causes primarily human
losis.
is: causes primarily bovine tuber-
.
croti: causes primarily tuberculosis
s.
um: causes primarily tuberculosis of

atuberculosis: causes Johne's disease
e.
ae: causes human leprosy.
aemurium: causes rat leprosy.

ococcus (My′ co.coc.cus) Kras-
v, 1938. *M p. 707.* Mikrobiologiya
R.), 7, Part I, 1938, 335.
ERENTIATING CHARACTERS: Free-
unicellular, nonsporing, nonphoto-
tic organisms; highly pleomorphic;
mainly as coccoid forms frequently
iable size and occasionally, especially
ids, as rod-shaped cells which are
tly branched. Large lemon-shaped
e not uncommon;* occur singly and
ins; motility not recorded; Gram-positive; nonacid-fast; *aerobic*; heterotrophic.

Type species: *Mycococcus albus* Krassilnikov.

NOTES: Six species are described. They are separated in the *Manual* on chromogenesis and action on milk.

Colonies are pigmented white, dark brown, red, orange, or yellow. In one species they are slimy and spreading.

Litmus milk is acidified and coagulated by one species, coagulated and peptonized by three, and unchanged by two.

Acid is produced from glucose and other carbohydrates by four species.

Citrate and acetate are metabolized by 2/4 species.

Starch is hydrolyzed by 2/4.

Sucrose is inverted by 3/4.

Nitrite is produced from nitrate by 2/3.

Paraffin is metabolized by 1/4 species.

Nocardia (No.car′ di.a) Trevisan, 1889. *M p. 713.* (I Generi e le Specie delle Batteriacee, 1889, 9.)

DIFFERENTIATING CHARACTERS: Nonphotosynthetic organisms which, during the early stages of growth, produce a well defined mycelium, less than 2 μ in width, which later fragments completely into branched or unbranched bacillary elements. When aerial mycelium is produced it also fragments into rods and coccal elements but does not produce differentiated conidia. The fragmentation may begin within a few hours or be delayed for several days; endospores not formed; *aerobic*; Gram-positive; heterotrophic; nonmotile (or if motile, they have flagella). Not known to deposit iron around the cells.

Type species: *Nocardia farcinica* Trevisan.

NOTES: The genus is represented by 40 species. Some species bear a resemblance to the genus *Arthrobacter*, the essential differentiating factor being the formation of a definite branching mycelium in the early stages of growth with species of *Nocardia*. In the subsequent disintegration

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sting cells formed in old cultures
ate like spores of actinomycetes.

of the mycelium extensive fragmentation may give rise to coccoid bodies which are similar in appearance to the aged cultures of *Arthrobacter*. Angulation of cells but no branching is observed in *Arthrobacter*.

According to Bisset and Moore (J. Gen. Microbiol., *3*, 1949, 387) the individual rods of *Nocardia* are multicellular and, when branching occurs, the branch is separated from the rod by a septum at the point of origin. This warrants further examination.

Subsequent growth of individual rods resulting from the fragmentation of the mycelium in *Nocardia* results in the formation of the zigzag pattern commonly found in microcolonies.

Certain species of *Nocardia* in which fragmentation is delayed may produce thickened aerial hyphae which abstrict cells from the tip, proceeding towards the base and finally involving the whole mycelium. Early stages in this change may be confused with *Streptomyces*, *e.g.*, *N. fordii* and *N. paraffinae*. Gordon (J. Bacteriol., *75*, 1958, 239) has described true conidia formation in *N. madurae*. If the contention of Bisset and Moore (J. Gen. Microbiol., *3*, 1949, 387) is correct, these species may, in fact, belong to the genus *Streptomyces*.

Pleomorphic forms are not uncommon. Swellings and bud-like processes are often produced.

There have been records of motility in some cultures of *Nocardia*.

Acid fastness occurs in some species, particularly in tissue lesions. It is infrequent in cultures.

The organisms grow well on laboratory media. Colonies are usually opaque, and white, yellow, orange, red, green, or black. They may be smooth and butyrous, or wrinkled and dough-like in consistency. The intensity of pigmentation varies with the medium employed.

Ammonia nitrogen is used by some species.

Gelatin is liquefied by 14/40 species.

Litmus milk is coagulated and pepton
by 8/40 and rendered alkaline and
tonized by 2/40; it is turned alkaline
by 6/40 and remains unchanged by
remainder. Pellicle formation and
mentation of the milk may occur.

Nitrite is produced from nitrate by
species.

Records of carbohydrate fermenta
and assimilation are very fragmenta
Seven of 13 species tested produce
but no gas from glucose. Of these only
produces acid from lactose.

Sixteen species are reported to metabo
paraffins, naphthalene, or phenols.

Two species, of marine origin, att
chitin, alginates, agar, and gum arabic.

Optimal temperatures lie between
and 37° C.

PATHOGENICITY: The following spe
are pathogenic to man and animals.

N. farcinica: bovine farcy.
N. asteroides: cerebral abscesses.
N. leishmanii: lung disease, pericarditis.
N. caprae: lesions in goats.
N. pretoriana: mycetoma of the chest wa
N. transvalensis: mycetoma of the foot.
N. madurae: mycetoma of the foot.
N. lutea: actinomycosis of the eye.
N. blackwellii: hock infection in foals.
N. rangoonensis: human pulmonary inf
tion.
N. caviae: lesions in guinea pigs.
N. gibsonii: acholuric jaundice.
N. africana: mycetoma of the foot.
N. fordii: spleen infection.

The only plant disease recorded is t
of gall of blueberry (*N. vaccinii*).

The nature and distribution of the
man and animal infections suggest tha
these species are soil inhabitants.

Actinomyces (Ac.ti.no.my′ ces) Ho
1877. *M p. 742.* (In Bollinger, Zentr. m
Wiss., *15*, 1877, 485.)

DIFFERENTIATING CHARACTERS: F
living, nonphotosynthetic organisms wh
in young cultures, produce a well defi
mycelium which fragments into simple
branched and branched rods as the cult

onmotile; no endospores produced;
positive; *anaerobic*; heterotrophic;
own to deposit iron about the cells.

species: *Actinomyces bovis*, Harz.

s: There are three species listed. *A.*
nd *A. israeli* produce a mycelium
disintegrates readily. That of *A.*
i is apparently more stable. In tis-
e terminal branches of the radiating
s are usually clubbed. This clubbed
is frequently acid-fast. In *A.*
it retains acid dyes. That of *A.*
i retains basic dyes.

rding to Bisset and Moore (J. Gen.
iol., *3*, 1949, 387) the branching
um of *Actinomyces* consists of uni-
rods which produce branches that
arated from the rods by a septum
point of origin. The authors contrast
th what they term 'true branching'
Streptomyces in which the branch is
parated at the point of origin. This
tion needs further examination.

et (J. Gen. Microbiol., *17*, 1957, 562)
ntends that some species of *Actino-*
produce conidia.

organisms are heterotrophic and
g. Colonies of *A. bovis* are small,
r, smooth, soft, and not adherent.
of *A. israeli* and *A. baudetii* are
and adherent to the medium.

quids growth occurs as small spheri-
fballs.

tin is not liquefied.

fer's inspissated serum is not lique-

d is not hemolyzed.

us milk may be acidified but rarely
ates.

l only is produced from glucose and
other carbohydrates.

erobic to microaerophilic.

imal temperature, 37° C.

true habitat is unknown. From the
f infection and history of cases all
probably occur in the soil or are
l inhabitants of body cavities.

ovis causes actinomycosis of cattle.

A. israeli causes actinomycosis in man and cattle.

A. baudetii causes actinomycosis of cats and dogs.

Jensenia (Jen.se′ ni.a) Bisset and Moore, 1950. (J. Gen. Microbiol., *4*, 1950, 280; also *3*, 1949, 387.)

DIFFERENTIATING CHARACTERS: Free-living, nonsporing, nonphotosynthetic, rod-shaped organisms, less than 2 μ wide, which exhibit rudimentary branching. Individual cells are unicellular and branches are separated from the parent cell by a septum at the point of origin; Gram-positive; nonacid-fast; *aerobic*; heterotrophic.

Type species: *Jensenia canicruria.*

NOTES: This genus is not recognized in the seventh edition of the *Manual.* There is only one species.

Rods and very short filaments containing one to four or five cells, each 1.0 to 1.2 μ by 5 to 10 μ; angular forms common; occasional short, impermanent branches.

Nonmotile; Gram-positive; nonacid-fast.

Obligate *aerobe*; optimal temperature, 30° to 33° C.

Slow growth on routine media, apparent after 48 hours.

Surface colonies circular, white, waxy, emulsifiable in saline; form a pellicle on fluid media. Do not ferment carbohydrates nor hydrolyze polysaccharides or proteins. Do not reduce nitrates. Capable of utilizing pyridine and allied cyclic compounds as sole carbon, nitrogen, and energy source.

Isolated from soil.

Streptomyces (Strep.to.my′ ces) Waksman and Henrici, 1943. *M p. 744.* (J. Bacteriol., *46*, 1943, 339.)

DIFFERENTIATING CHARACTERS: Free-living, filamentous, nonphotosynthetic, rod-shaped organisms, less than 2 μ in width, which in young cultures produce a branching mycelium that does not disintegrate entirely as the culture ages. Chains of conidia are produced from aerial hyphae; sporangia are not produced; nonmotile; endospores not produced; *aerobic*; Gram-positive; heterotrophic.

Type species: *Streptomyces albus* (Rossi-Doria, emend. Krainsky)Waksman and Henrici.

NOTES: a. Cultures of *Actinoplanes* or *Streptosporangium* on some laboratory media are morphologically similar to *Streptomyces.*

b. Certain species of *Nocardia* in which fragmentation is delayed may produce thickened aerial hyphae which abstrict cells from the tip, proceeding towards the base and finally involving the whole mycelium. Early stages in this change may be confused with *Streptomyces, e.g., N. fordii* and *N. paraffinae.*

The *Manual* lists 150 species. Apart from morphological and cultural characters, information on the species is exceedingly fragmentary. Germination of conidia may be unipolar or multipolar. In the early stages of development a radiating mycelium is produced which stains uniformly Gram-positive. Side branches in some species are of the same width as the main filament. In others a bud-like outgrowth attached to the main mycelium by a very thin stalk grows out to form the side branch. According to Bisset and Moore (J. Gen. Microbiol., *3,* 1949, 387) the mycelium consists of a number of unicellular components which branch without producing a septum at the point where the branch leaves the mother cell.

When aerial hyphae are produced, or in old colonies, degeneration occurs and the mycelium does not stain uniformly.

The conidia producing branches have characteristic shapes and arrangements which are used for purposes of separation. They may occur at random or in clusters. They may be unbranched; monopodially or dichotomously branched or branching may occur in the form of whorls on the main aerial hypha. The terminal portion from which the conidia are produced may be straight or spiral. Spiral forms are open or compact and may be of the sinistrorse (left hand spiral) or dextrorse (right hand spiral) type.

The production of conidia results f
the reorganization of the cytoplasm *wi*
the aerial hypha, and the chain of con
is produced within and finally separ
from the original wall of the aerial hyph

Conidia may be spherical, oval,
cylindrical, smooth or sculptured. T
formation usually results in the surfac
colonies becoming powdery and dry.
conidial layer frequently has a differ
pigmentation from the main myceli
Conidia stain intensely Gram-positive
sharp contrast to the almost Gram-ne
tive appearance of the aged mycelium fr
which the conidiophores arise. Conidia
nonmotile. Chlamydospores are frequen
produced in the mycelium proper.

No clear statement appears in
Manual regarding the nutritional requ
ments of the *Streptomyces.* Many spe
are capable of growth on synthetic me
with ammonia nitrogen, and they are
infrequently found as contaminants
organic carbon-free media. The appeara
of the colonies varies with the type
medium employed. In all media upon wh
growth occurs the mycelium penetra
into the agar, and the initial colonies
usually circular and partly submerged a
have a dull waxy appearance. The agar
frequently depressed around the margin
the colony. If no aerial mycelium is form
the colony may continue to develop
this form. Where aerial mycelium is p
duced the surface of the colony becon
floccose and, with the production of
conidia, eventually powdery.

Stress within the developing colon
frequently causes apparent splitting of
colonies. In some species droplets of gut
tion water appear on the surface of
colony and upon drying out leave craters
the surface.

Most species have a pronounced eart
odor. Many produce pigments, either

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* T. R. Vernon, Actinomycetes in N
Zealand Soils. Paper read at the Congress
the Australian and New Zealand Associat
for Advancement of Science, Dunedin, 19

ILLUSTRATIONS

e following illustrations have been compiled from several sources for the purpose of
ing the cardinal features of genera mentioned in the Keys and in the *Manual*. A
number of them are original photomicrographs from slide cultures prepared by the
niques described in Methods.

ith the following exceptions, all organisms photographed by phase contrast have
cultured on peptone yeast-extract agar containing 0.5 per cent sodium chloride.
robium: Chlorobium medium.
obacter, Diplococcus, Streptococcus, Pediococcus, Leuconostoc, Lactobacillus, Propioni-
cterium: peptone yeast-extract agar + 1 per cent glucose.
obacter: 0.1 per cent peptone water.
illum: Williams and Rittenberg's *Spirillum* medium.
bacterium: Gibbons' halophile agar.
obacterium: fish agar.
erotilus: 0.1 per cent peptone-iron medium.
mophilus: Levinthal + X- and V-factor agar.
ptomyces: Nitrosomonas mineral salts agar. (The growth of the *Streptomyces* was
oded with acetone and immediately covered with a coverslip before being photo-
raphed.)

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oth, and their courtesy is very gratefully acknowledged.

here have been a few omissions caused mainly by lack of suitable cultures for the
trations.

is hoped that these illustrations will help materially in the interpretation of the Keys.

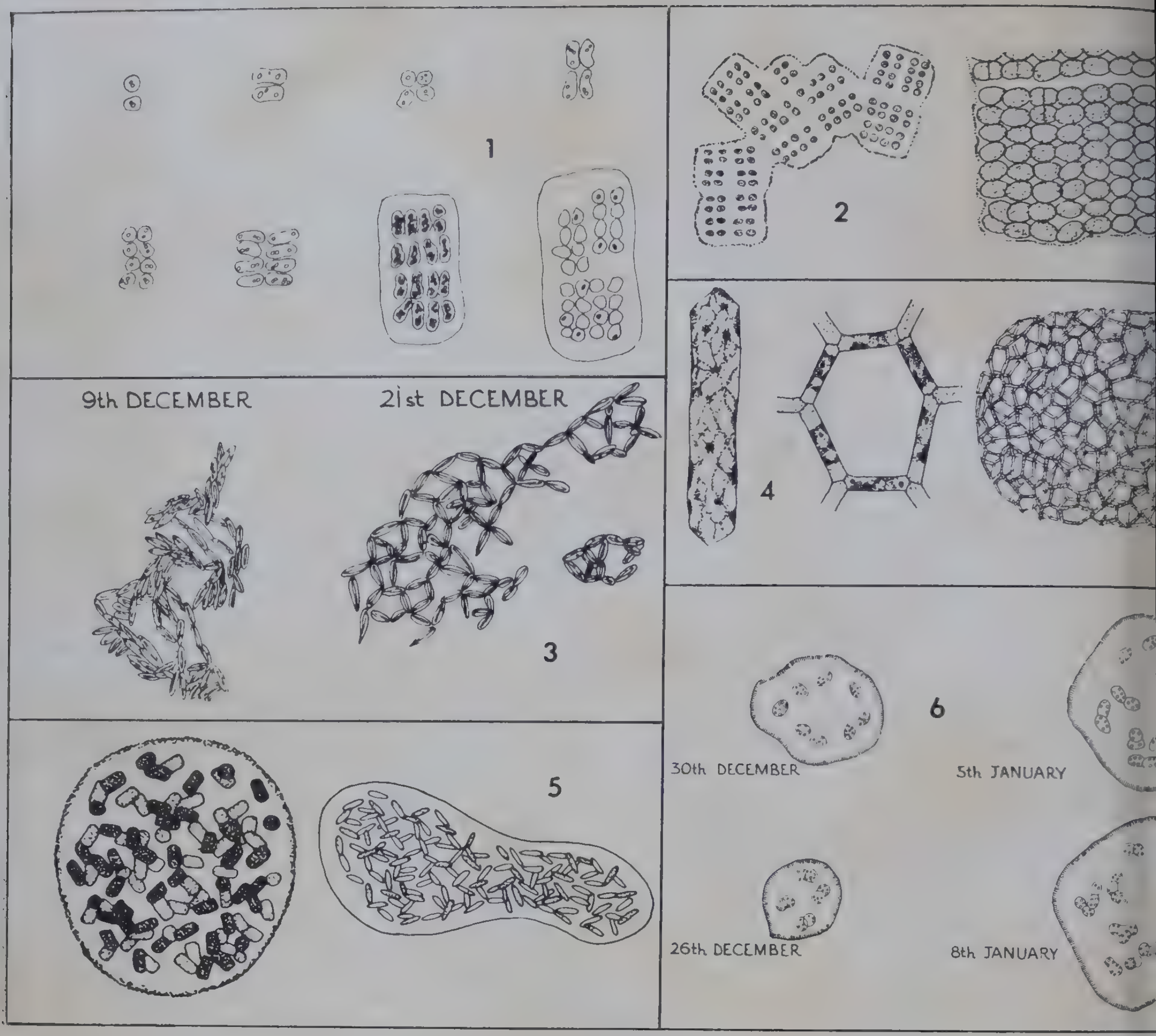

1
2
9th DECEMBER
21st DECEMBER
3
4
5
6
30th DECEMBER
5th JANUARY
26th DECEMBER
8th JANUARY

PLATE 1

1. *Thiopedia rosea.* Reproduced by permission from Winogradsky, Microbiologie d Masson et Cie, Paris. × 1000. 2. *Merismopedia* sp. Reproduced by permission from Smith, Freshwater Algae of the United States, McGraw-Hill Book Company. × 3 *Thiodictyon elegans.* Reproduced by permission from Winogradsky, Microbiologie d Masson et Cie, Paris. × 450. 4. *Hydrodictyon* sp. Reproduced by permission from Smith, Freshwater Algae of the United States, McGraw-Hill Book Company. × 2 *Aphanothece* sp. Reproduced by permission from G. M. Smith, Freshwater Algae o United States, McGraw-Hill Book Company. × 430. 6. *Thiothece gelatinosa.* Reproduc permission from Winogradsky, Microbiologie du Sol, Masson et Cie, Paris. × 320.

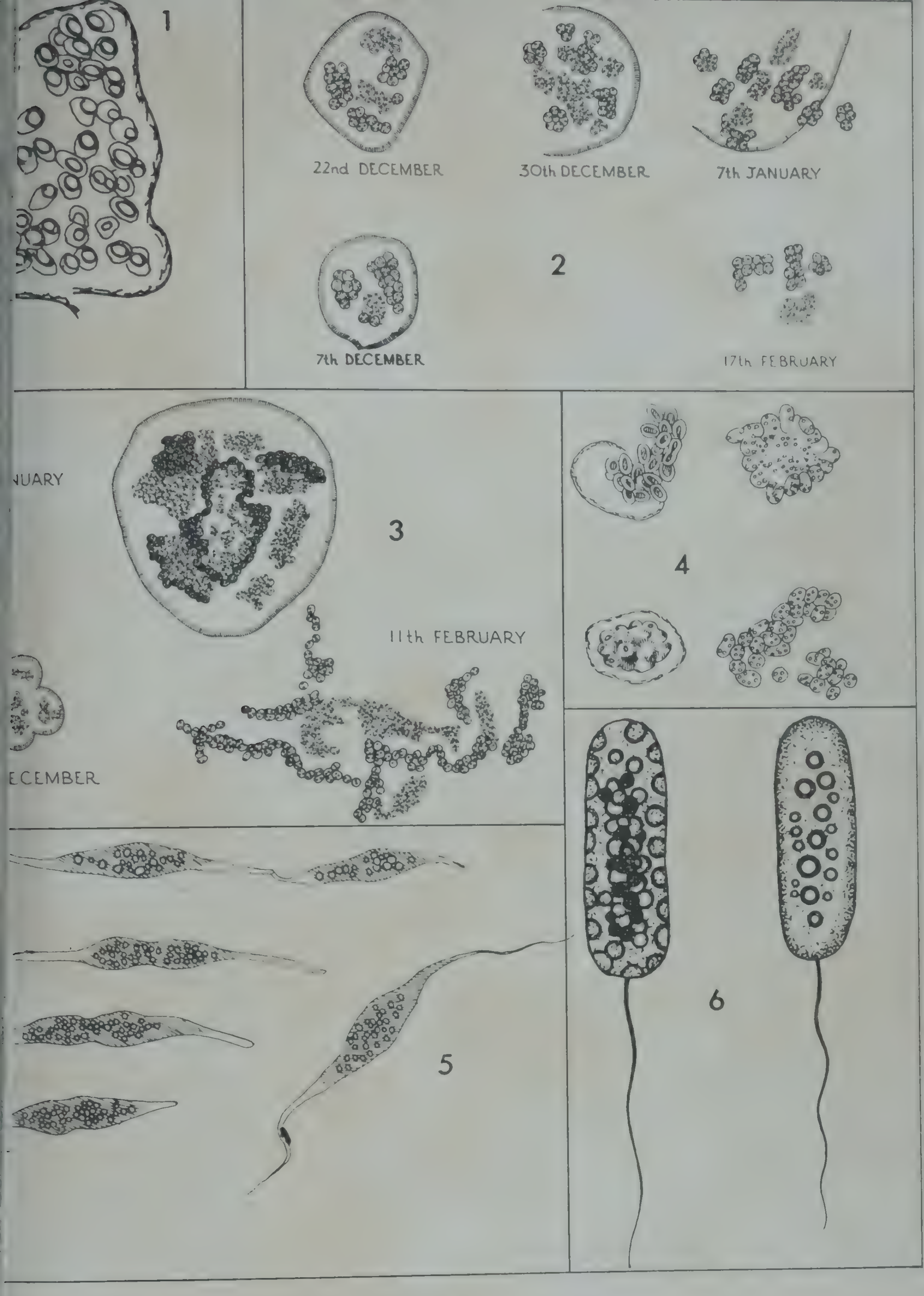

1
22nd DECEMBER
30th DECEMBER
7th JANUARY
2
7th DECEMBER
17th FEBRUARY
NUARY
3
11th FEBRUARY
ECEMBER
4
5
6

PLATE 2

. *Thiocapsa roseo-persicina.* Reproduced by permission from Winogradsky, Microbio-
du Sol, Masson et Cie, Paris. × 1050. **2.** *Thiocystis violacea.* Reproduced by permission
Winogradsky, Microbiologie du Sol, Masson et Cie, Paris. × 420. 3. *Lamprocystis*
o-persicina. Reproduced by permission from Winogradsky, Microbiologie du Sol, Mas
et Cie, Paris. × 420. **4.** *Amoebobacter roseus.* Reproduced by permission from Wino-
dsky, Microbiologie du Sol, Masson et Cie, Paris. × 700. **5.** *Rhabdomonas roseum.* Re-
duced by permission from Winogradsky, Microbiologie du Sol, Masson et Cie, Paris.
00. **6.** *Chromatium linsbaueri.* From Gicklhorn, Ber. deut. botan. Ges., *39*, 1921, 312.
urtesy of Gustav Fischer, Stuttgart. × 2550.

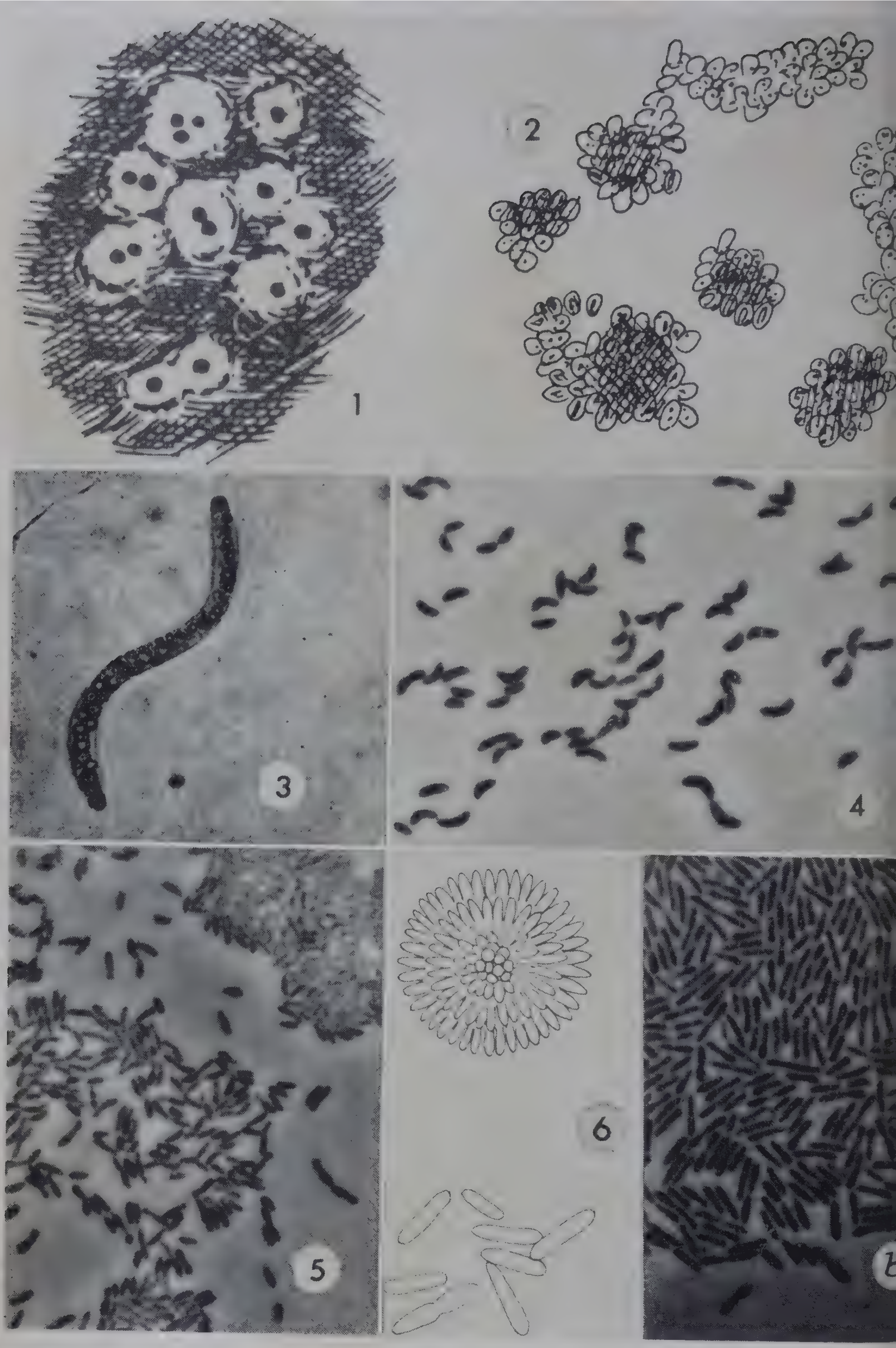


PLATE 3

1. *Rhodothece pendens*. Reproduced by permission from Ellis, The Sulphur Bact
Longmans, Green and Co. Limited. × 1000. 2. *Thiopolycoccus ruber*. Reproduced by
mission from Winogradsky, Microbiologie du Sol, Masson et Cie, Paris. × 1400. 3.
spirillum sp. From Omelianski, Zentr. Bakteriol. Parasitenk., Abt. II, Bd. XIV. Cou
of Gustav Fischer, Stuttgart. × 500. 4. *Rhodospirillum rubrum*. Slide culture. Orig
× 1800. 5. *Rhodopseudomonas sphaeroides*. Slide Culture. Original. × 1800. 6. *Van*
aggregata. *a*, Reproduced by permission from Pringsheim, J. Gen. Microbiol., *13*, 1955,
× 1000. *b*, Slide culture. Original. × 1800.

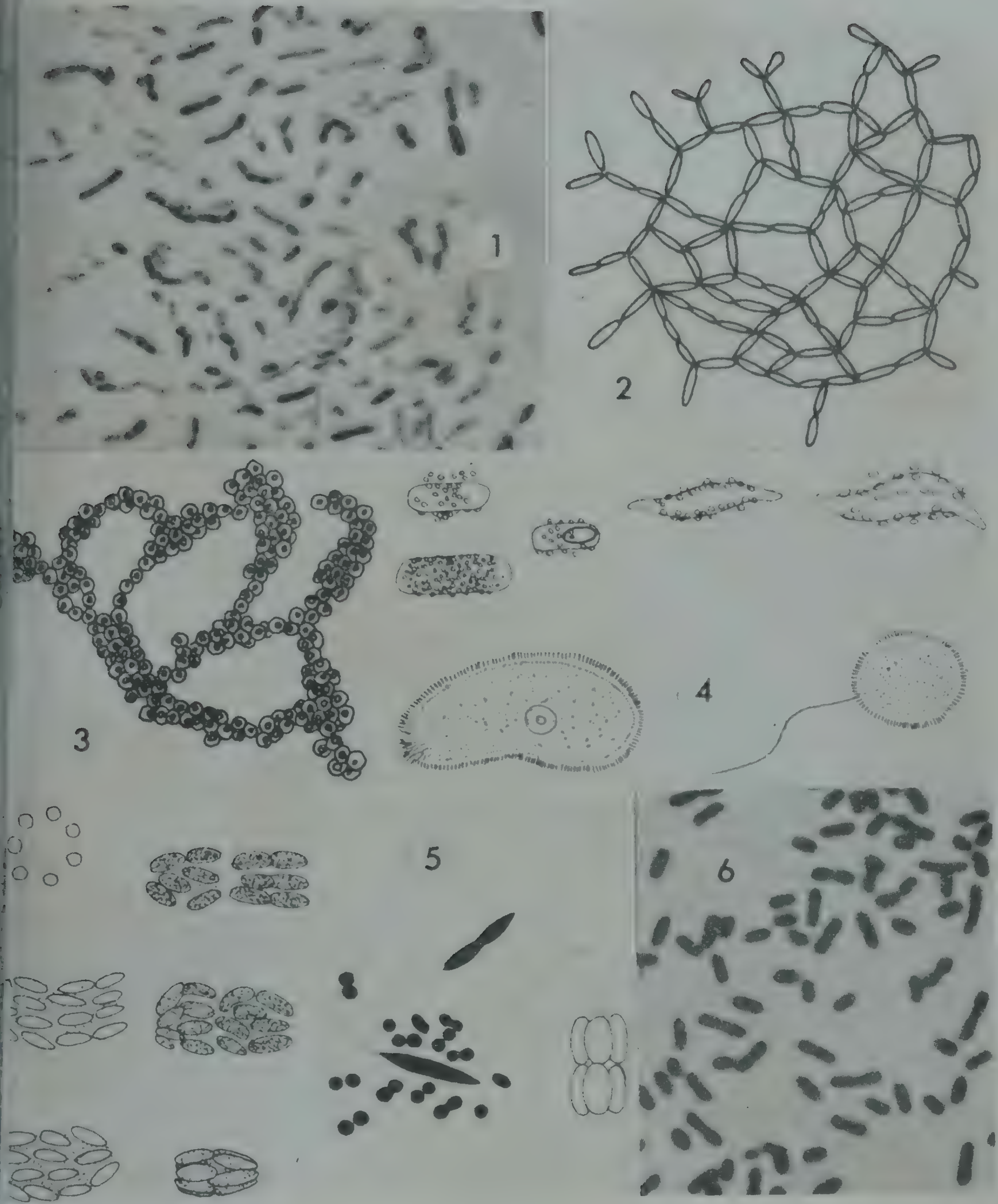


PLATE 4

1. *Chlorobium* sp. Flask culture. Original. × 1800. **2.** *Pelodictyon* sp. From Geitler and
scher, Die Süsswasserflora Deutschlands, Österreichs und der Schweiz, Jena, *12*, 1925,
. Courtesy of Gustav Fischer, Stuttgart. × 1000. **3.** *Clathrochloris sulphurica*. From
itler and Pascher, Die Süsswasserflora Deutschlands, Österreichs und der Schweiz, Jena,
. 1925, 457. Courtesy of Gustav Fischer, Stuttgart. × 2000. **4.** *Chlorobacterium symbioticum*.
om Geitler and Pascher, Die Süsswasserflora Deutschlands, Österreichs und der Schweiz,
na, *12*, 1925, 462. Courtesy of Gustav Fischer, Stuttgart. × 1000. **5.** *Chlorochromatium*
regatum. After Lauterborn and after Buder, from Geitler and Pascher, Die Süsswasser-
ra Deutschlands, Österreichs und der Schweiz, Jena, *12*, 1925, 459. Courtesy of Gustav
scher, Stuttgart. × 1000. **6.** *Nitrosomonas* sp. Reproduced by permission from Winograd
y, Microbiologie du Sol, Masson et Cie, Paris. × 1800.

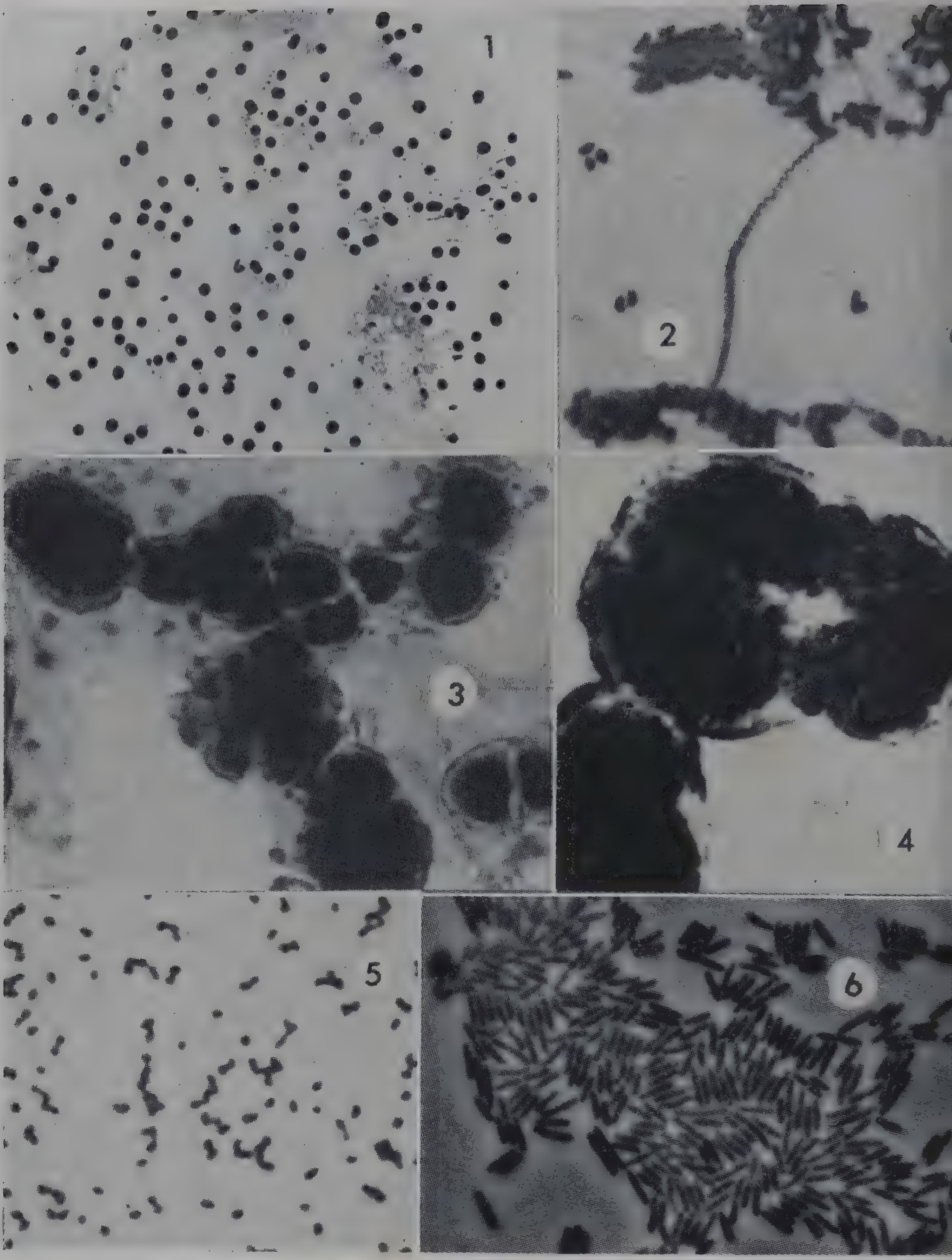


PLATE 5

1. *Nitrosococcus nitrosus.* Reproduced by permission from Winogradsky, Microbiol
du Sol, Masson et Cie, Paris. × 1800. 2. *Nitrosospira* sp. Reproduced by permission f
Winogradsky, Microbiologie du Sol, Masson et Cie, Paris. × 1800. 3. *Nitrosocystis* sp.
produced by permission from Winogradsky, Microbiologie du Sol, Masson et Cie, P
× 1800. 4. *Nitrosogloea* sp. Reproduced by permission from Winogradsky, Microbiol
du Sol, Masson et Cie, Paris. × 1800. 5. *Nitrobacter* sp. Reproduced by permission f
Winogradsky, Microbiologie du Sol, Masson et Cie, Paris. × 1800. 6. *Hydrogenomona*
Slide culture. Original. × 1800.

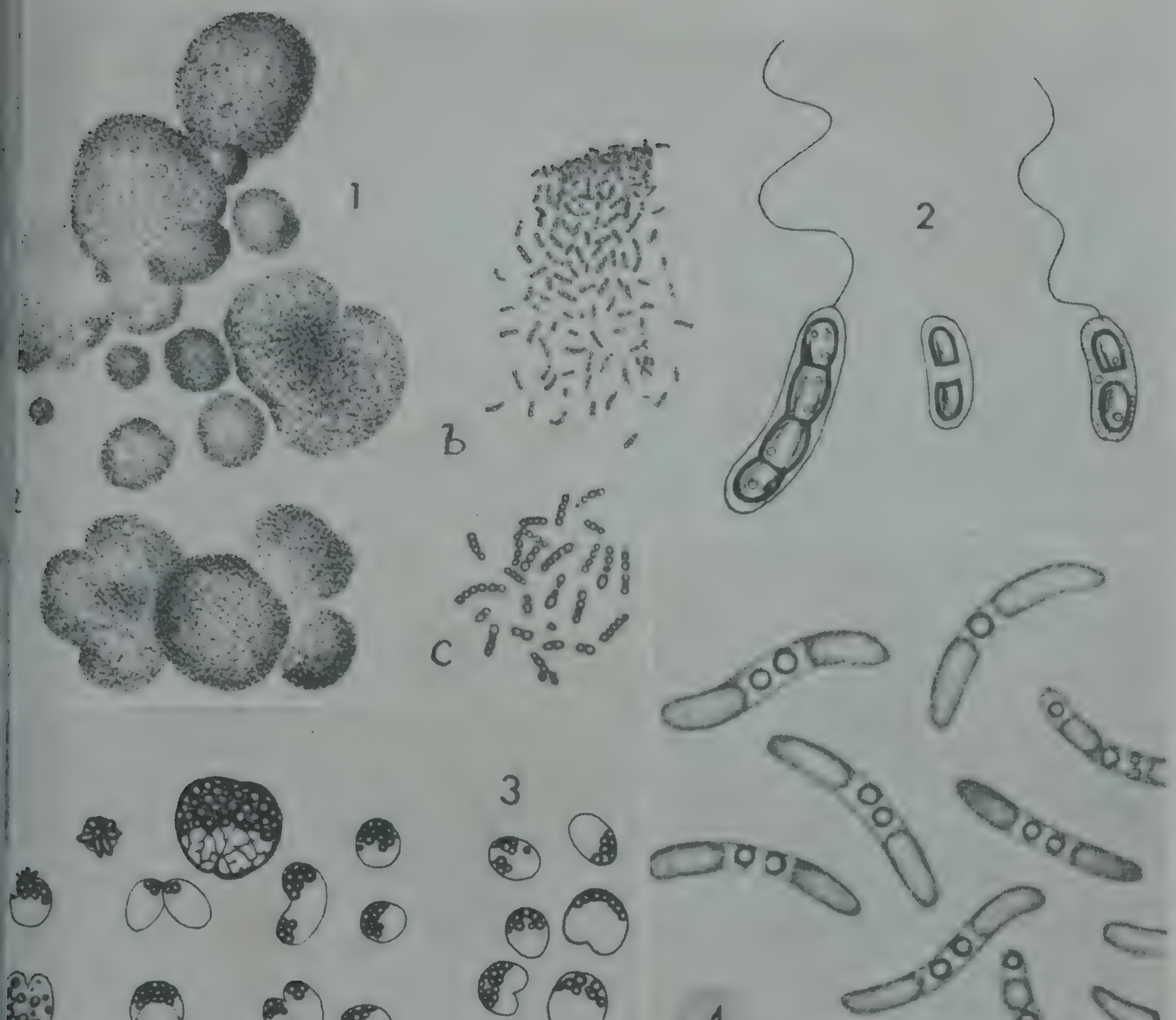


PLATE 6

1. *Thiobacterium bovista.* From Molisch, Zentr. Bakteriol. Parasitenk., Abt. II, *33*, 1912,
. Courtesy of Gustav Fischer, Stuttgart. *a*, × 1000, *b*, × 1000, *c*, × 1000. **2.** *Macromonas
obilis.* Reproduced by permission from Ellis, The Sulphur Bacteria. Longmans, Green
nd Co. Limited. × 1000. **3.** *Thiovulum majus.* From Hinze, Ber. deut. botan. Ges., *31*,
13, 195. Courtesy of Gustav Fischer, Stuttgart. × 750. **4.** *Thiospira bipunctatum.* From
olisch, Zentr. Bakteriol. Parasitenk. Abt. II, *33*, 1912, 55. Courtesy of Gustav Fischer,
uttgart. × 1500. **5.** *Thiobacillus thiooxidans.* Line drawing from photomicrograph repro-
uced by permission from Knaysi, J. Bacteriol., *46*, 1943, 455. × 10,000. **6.** *Pseudomonas
ruginosa.* Slide culture. Original. × 1800.

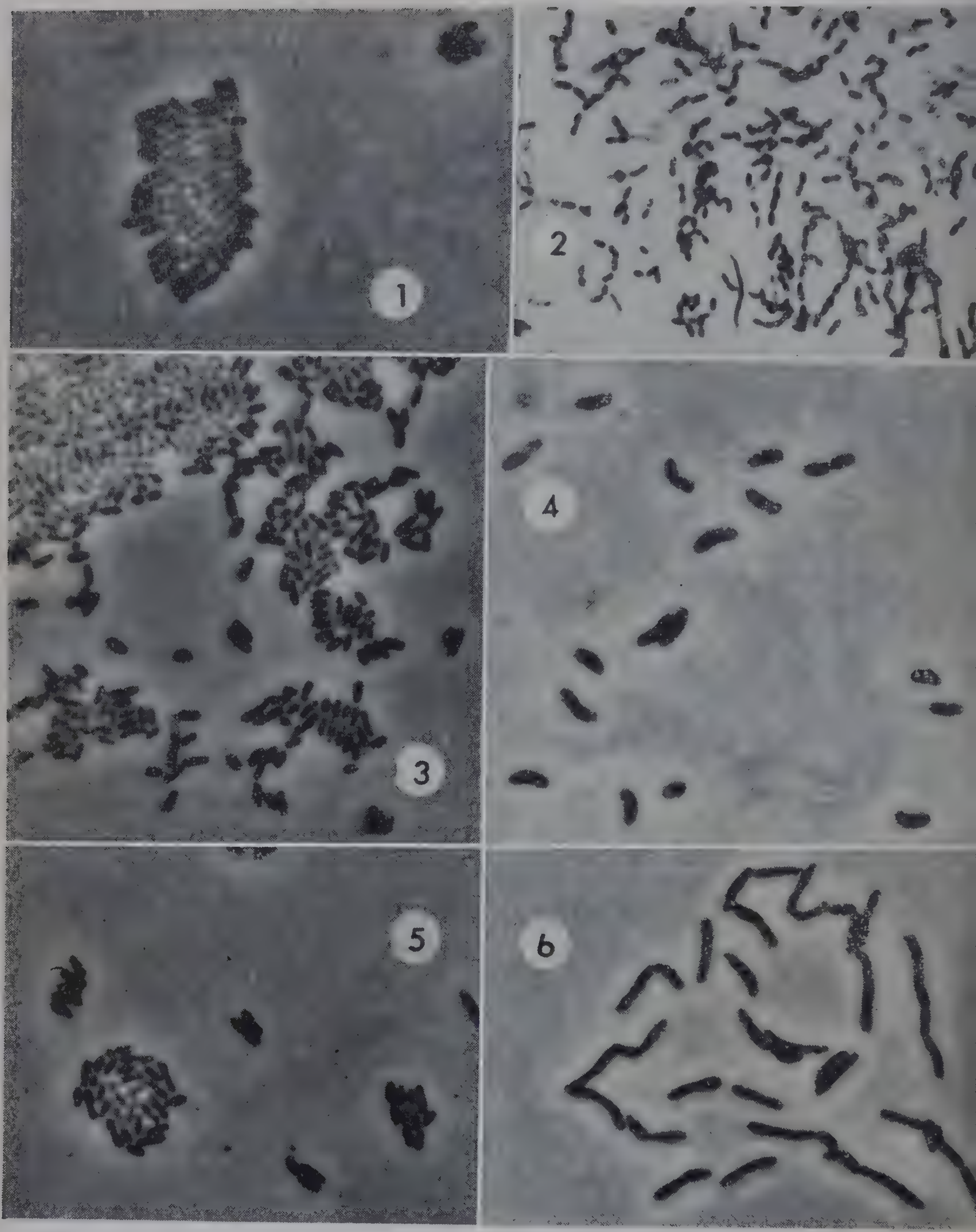


Plate 7

1. *Xanthomonas phaseoli*. Slide culture. Original. × 1800. 2. *Acetobacter* sp. Slide cult Original. × 1800. 3. *Aeromonas* sp. Slide culture. Original. × 1800. 4. *Photobacterium* Slide culture. Original. × 1800. 5. *Azotomonas insolita*. Slide culture. Original. × 1 6. *Protaminobacter ruber*. Slide culture. Original. × 1800.

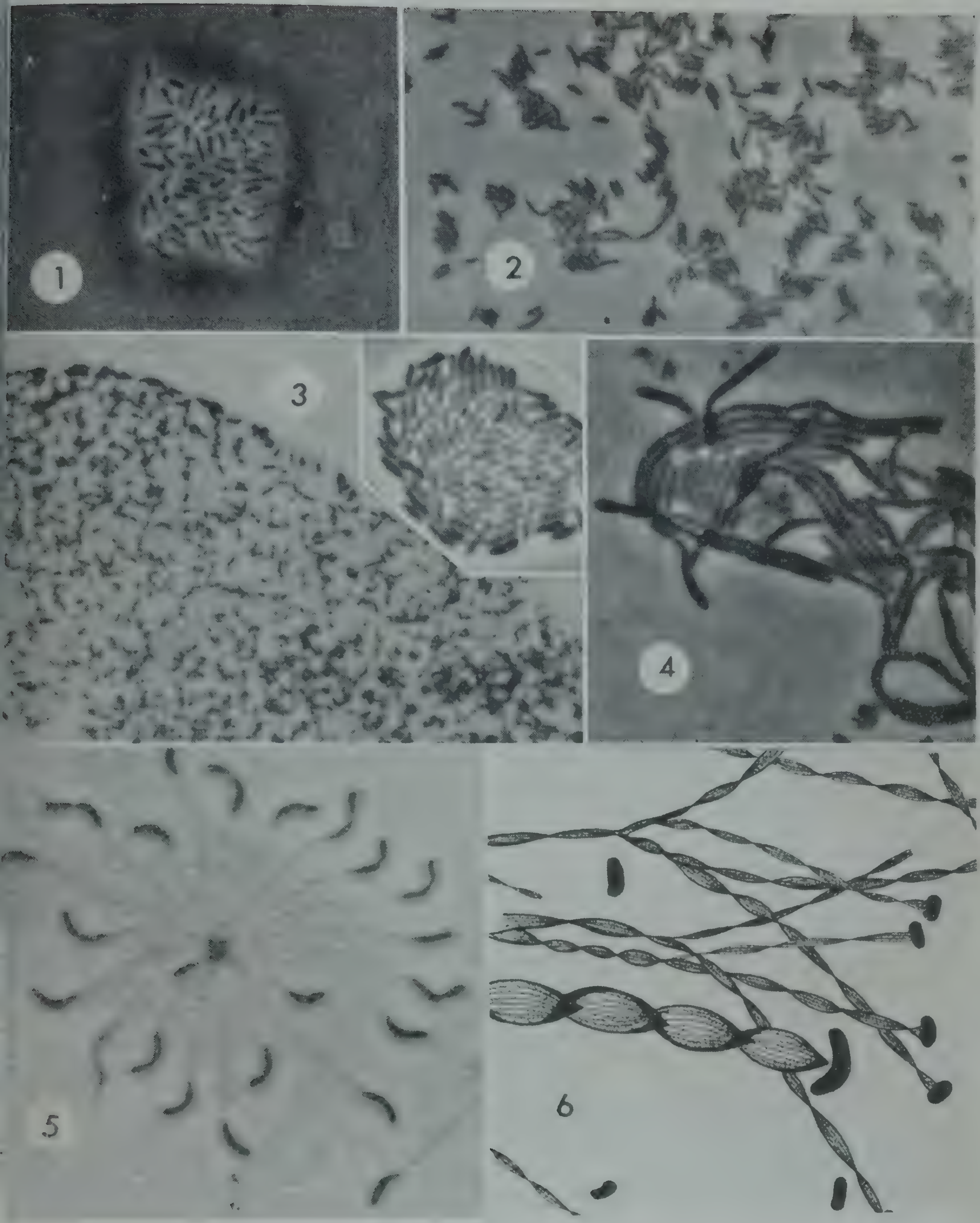


PLATE 8

1. *Alginomonas* sp. Slide culture. Original. × 1800. **2.** *Mycoplana bullata*. Slide culture.
iginal. × 1800. **3.** *Zoogloea ramigera*. Lobe from natural growth showing dispersed bac-
rial cells. Original. × 840. *Inset*, Growth on slide culture peptone-yeast extract agar.
iginal. × 1800. **4.** *Halobacterium* sp. From 16 per cent salt agar. Original. × 1800. **5.** *Cau-*
acter sp. From 0.1 per cent peptone. Original. × 1800. **6.** *Gallionella* sp. Line drawing
om photomicrograph by Cholodny, taken from Dorff, Die Eisenorganismen, Pflanzen-
rschung, Heft *16*, 1934, 25. Courtesy of Gustav Fischer, Stuttgart. × 1950.

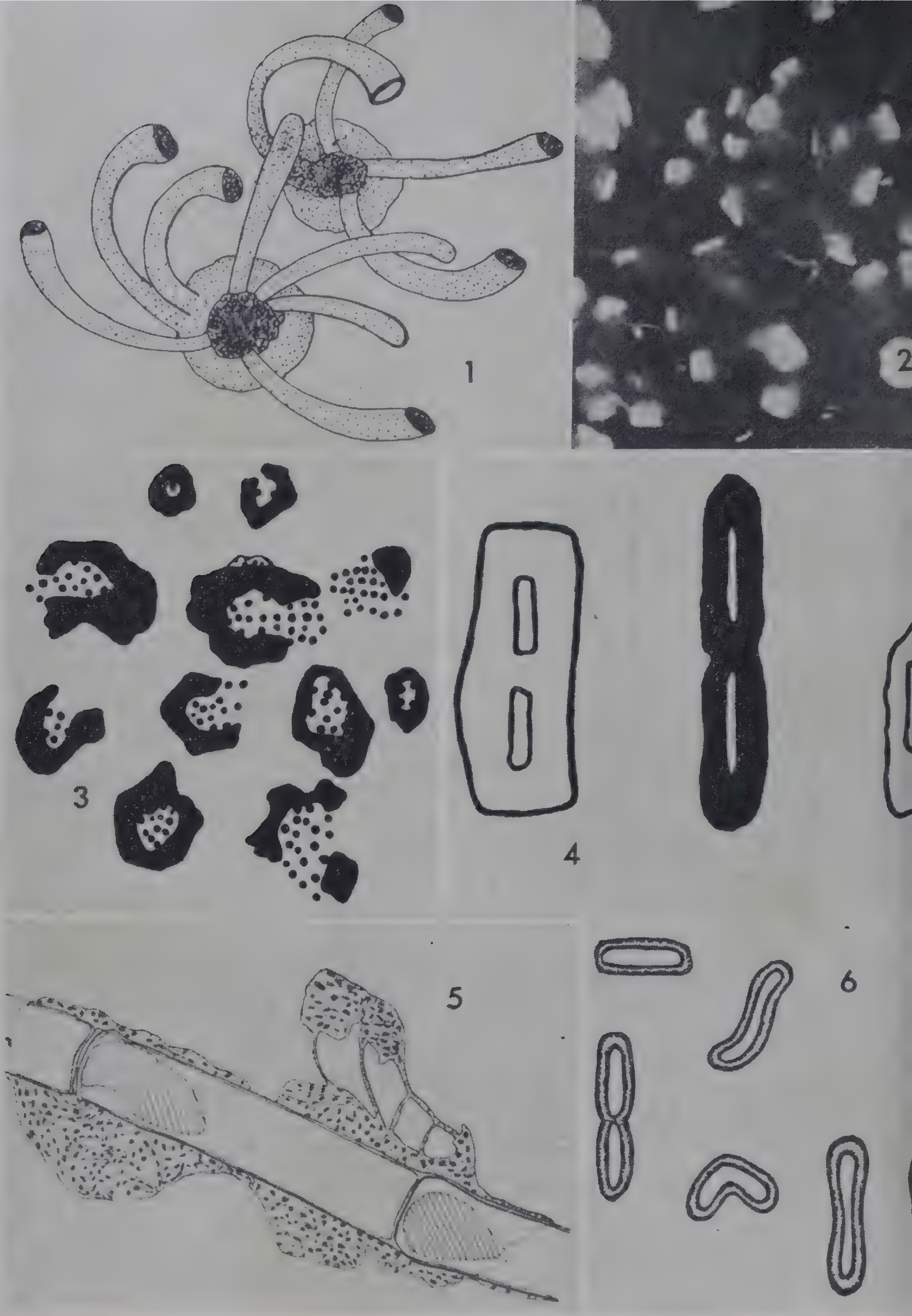


PLATE 9

1. *Siderophacus corneolus.* From Dorff, Die Eisenorganismen, Pflanzenforschung,
16, 1934, 26. Courtesy of Gustav Fischer, Stuttgart. × 2250. **2.** *Nevskia ramosa.* Phot
crograph reproduced by permission from Henrici and Johnson, J. Bacteriol., *30*, 1935
× 500. **3.** *Siderocapsa major.* From Molisch, taken from Dorff, Die Eisenorganismen, P
zenforschung, Heft *16*, 1934, 15. Courtesy of Gustav Fischer, Stuttgart. × 2000. **4.** *F*
bacterium rectangulare. From Naumann, taken from Dorff, Die Eisenorganismen, Pflan
forschung, Heft *16*, 1934, 15. Courtesy of Gustav Fischer, Stuttgart. × 3000. **5.** *Siderom*
confervarum on Oedogonium. From Dorff, Die Eisenorganismen, Pflanzenforschung,
16, 1934, 14. Courtesy of Gustav Fischer, Stuttgart. × 900. **6.** *Naumanniella neusto*
From Dorff, Die Eisenorganismen, Pflanzenforschung, Heft *16*, 1934, 20. Courtesy of
tav Fischer, Stuttgart. × 2000.

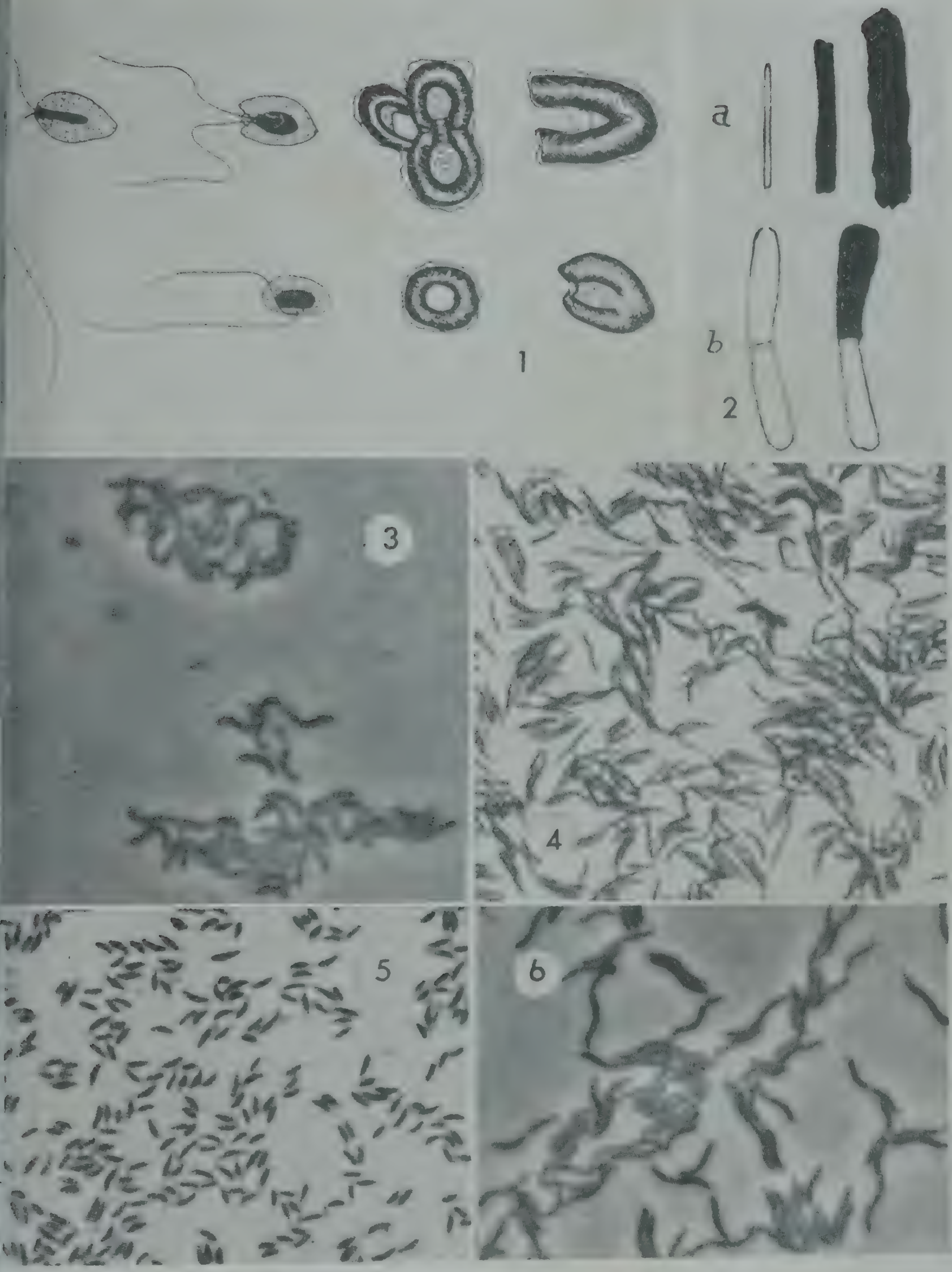


PLATE 10

1. *Ochrobium tectum*. From Dorff, Die Eisenorganismen, Pflanzenforschung, Heft *16*, 34, 18. Courtesy of Gustav Fischer, Stuttgart. × 2000. **2.** *Siderobacter* sp. *a*, *S. lineare*, *S. duplex*. After Naumann, from Dorff, Die Eisenorganismen, Pflanzenforschung, Heft 1934, 16. Courtesy of Gustav Fischer, Stuttgart. × 2000. **3.** *Vibrio metschnikovii*. Slide ture. Original. × 1800. **4.** *Cellvibrio flavescens*. Reproduced by permission from Winodsky, Microbiologie du Sol, Masson et Cie, Paris. × 1850. **5.** *Cellfalcicula fusca*. Reproed by permission from Winogradsky, Microbiologie du Sol, Masson et Cie, Paris. 1850. **6.** *Spirillum itersonii*. Slide culture. Original. × 1800.

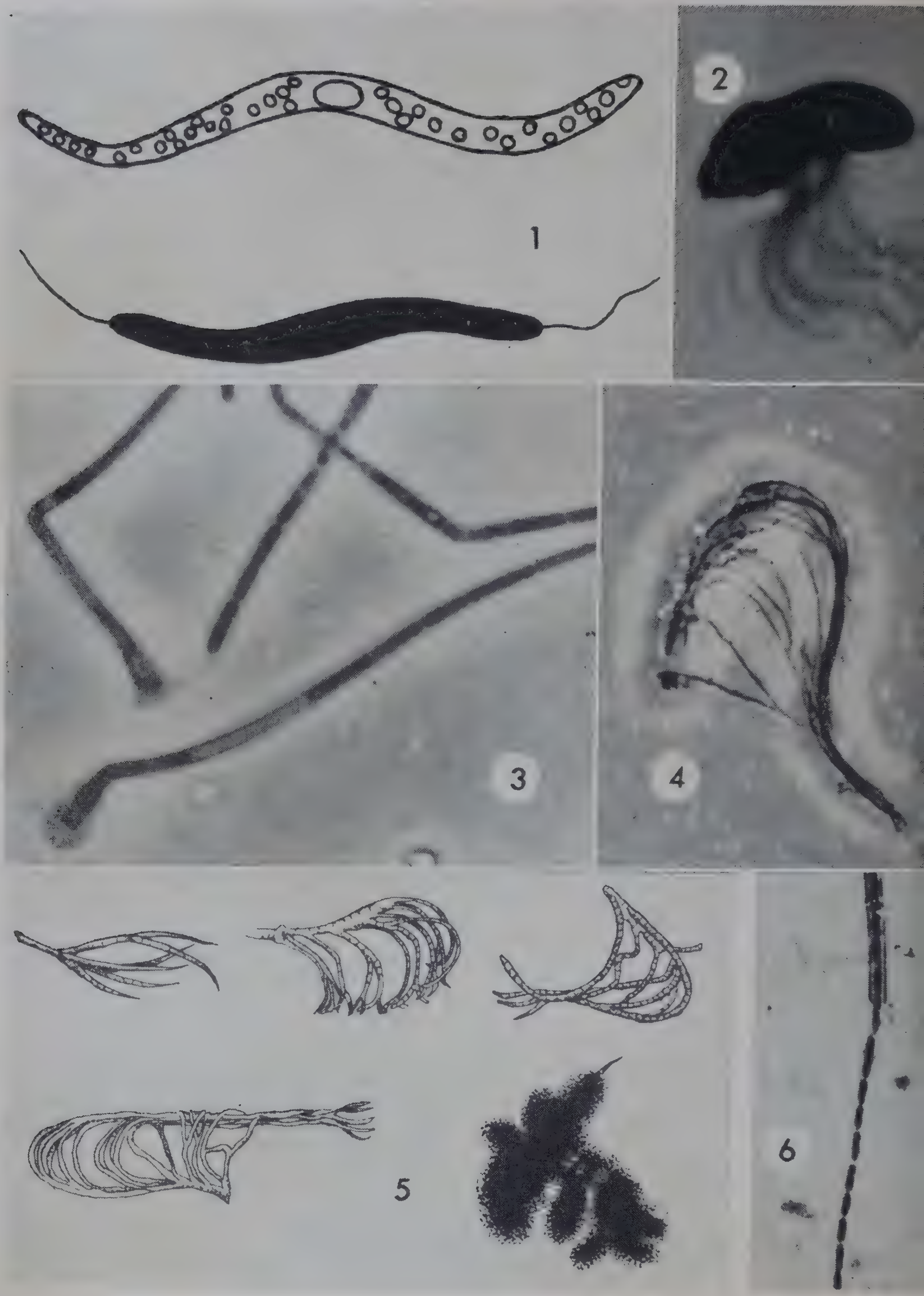


Plate 11

1. *Paraspirillum vejdovskii*. From Dobell, Arch. Protistenk., *24*, 1912, 97. Courte Gustav Fischer, Stuttgart. × 4000. 2. *Selenomonas* sp. Courtesy of C. F. Robinow. × 3. *Sphaerotilus natans*. From iron peptone water. Original. × 1800. 4. *Toxothrix trichog* Original. × 1800. 5. *Toxothrix trichogenes*. From Molisch, taken from Dorff, Die Eise ganismen, Pflanzenforschung, Heft *16*, 1934, 39. Courtesy of Gustav Fischer, Stuttg × 660. 6. *Leptothrix ochracea*. From Cholodny, Die Eisenbakterien, Pflanzenforsch Heft *16*, 1934, Table 4, fig. 20. Courtesy of Gustav Fischer, Stuttgart. × 1000.

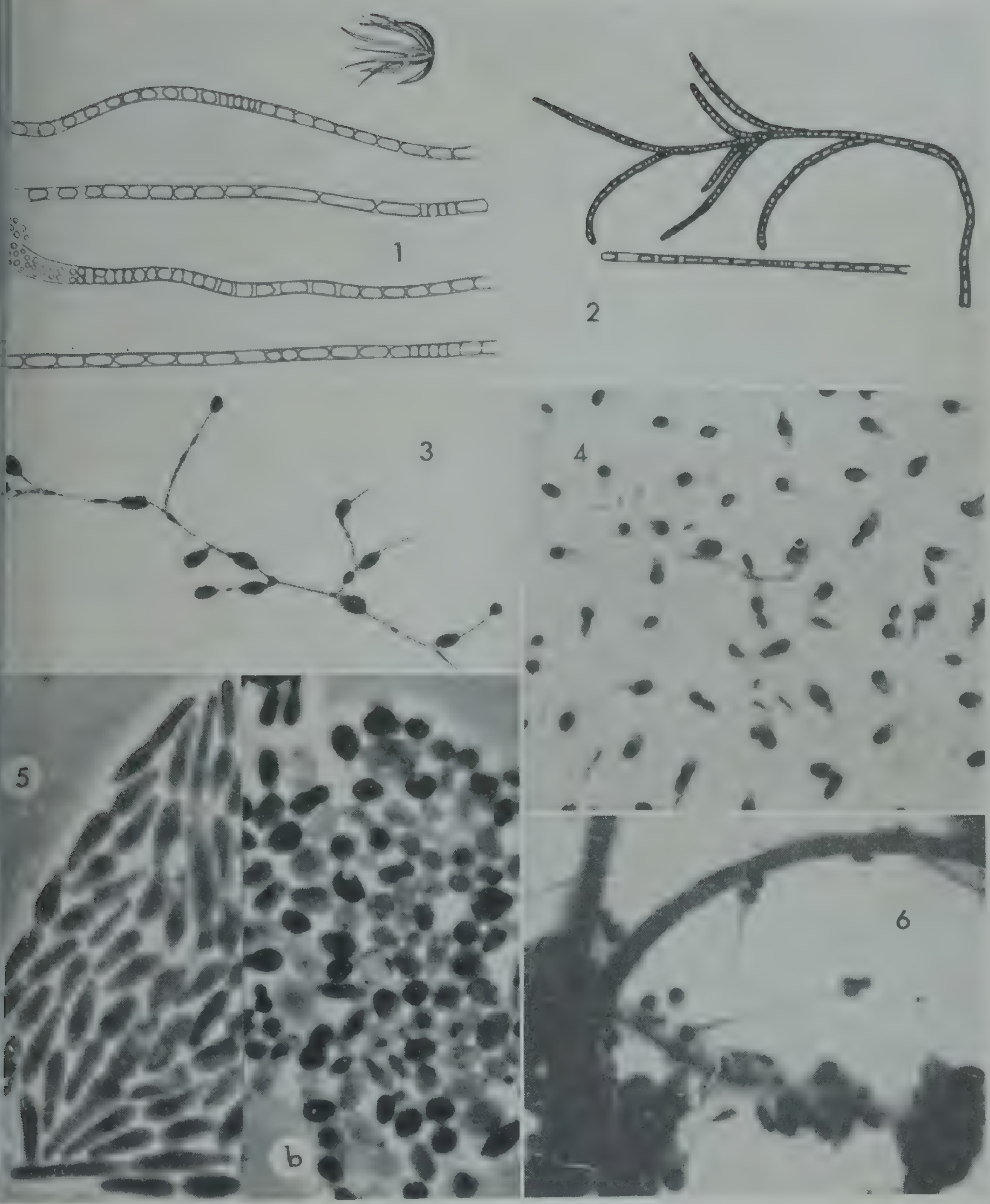


PLATE 12

Crenothrix polyspora. From Dorff, Die Eisenorganismen, Pflanzenforschung, Heft *16*,
, 40. Courtesy of Gustav Fischer, Stuttgart. × 1100. **2.** *Clonothrix fusca*. From Molisch,
en from Dorff, Die Eisenorganismen, Pflanzenforschung, Heft *16*, 1934, 41. Courtesy of
stav Fischer, Stuttgart. × 750. **3.** *Rhodomicrobium vannielii*. Line drawing of photo-
rograph reproduced by permission from Duchow and Douglas, J. Bacteriol., *58*, 1949,
. × 850. **4.** *Pasteuria ramosa*. Photomicrograph reproduced by permission from Henrici
Johnson, J. Bacteriol., *30*, 1935, 93. × 850. **5.** *Azotobacter chroococcum*. *a*, Early rod
nge stage; *b*, early spherical stage. Originals. × 1500. **6.** *Blastocaulis sphaerica*. Photo-
rograph reproduced by permission from Henrici and Johnson, J. Bacteriol., *30*, 1935,
× 1260.

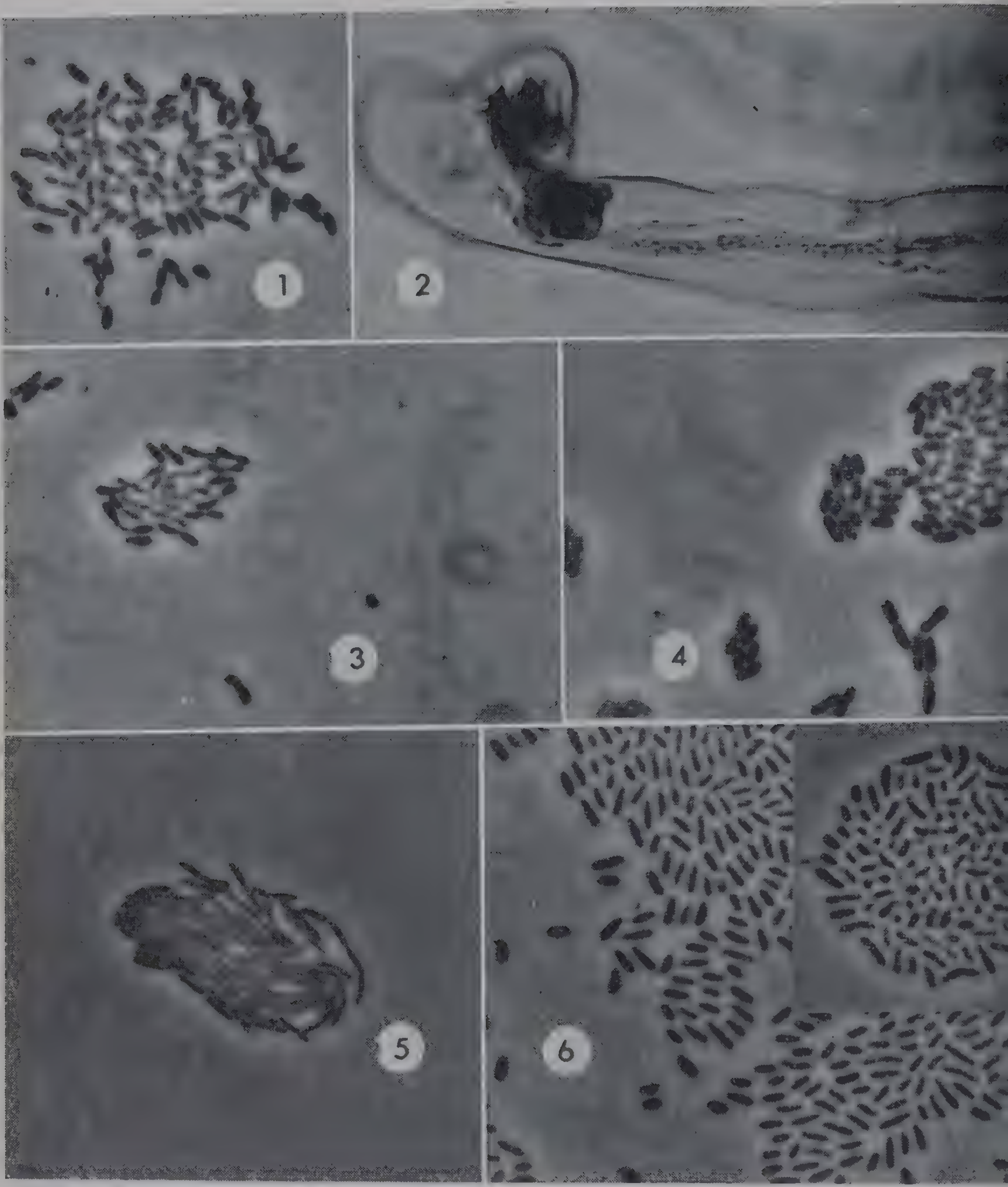


Plate 13

1. *Rhizobium* sp. Slide culture. Original. × 1620. **2.** Infection thread of *Rhizobium* root hair of a legume. Courtesy of Gösta Fåhraeus. × 1620. **3.** *Agrobacterium tumef* Slide culture. Original. × 1620. **4.** *Chromobacterium violaceum*. Slide culture. Origi 1620. **5.** *Alcaligenes faecalis*. Slide culture. Original. × 1620. **6.** *Agarbacterium* sp. Dis cells. *Inset,* microcolony. Original. × 1620.

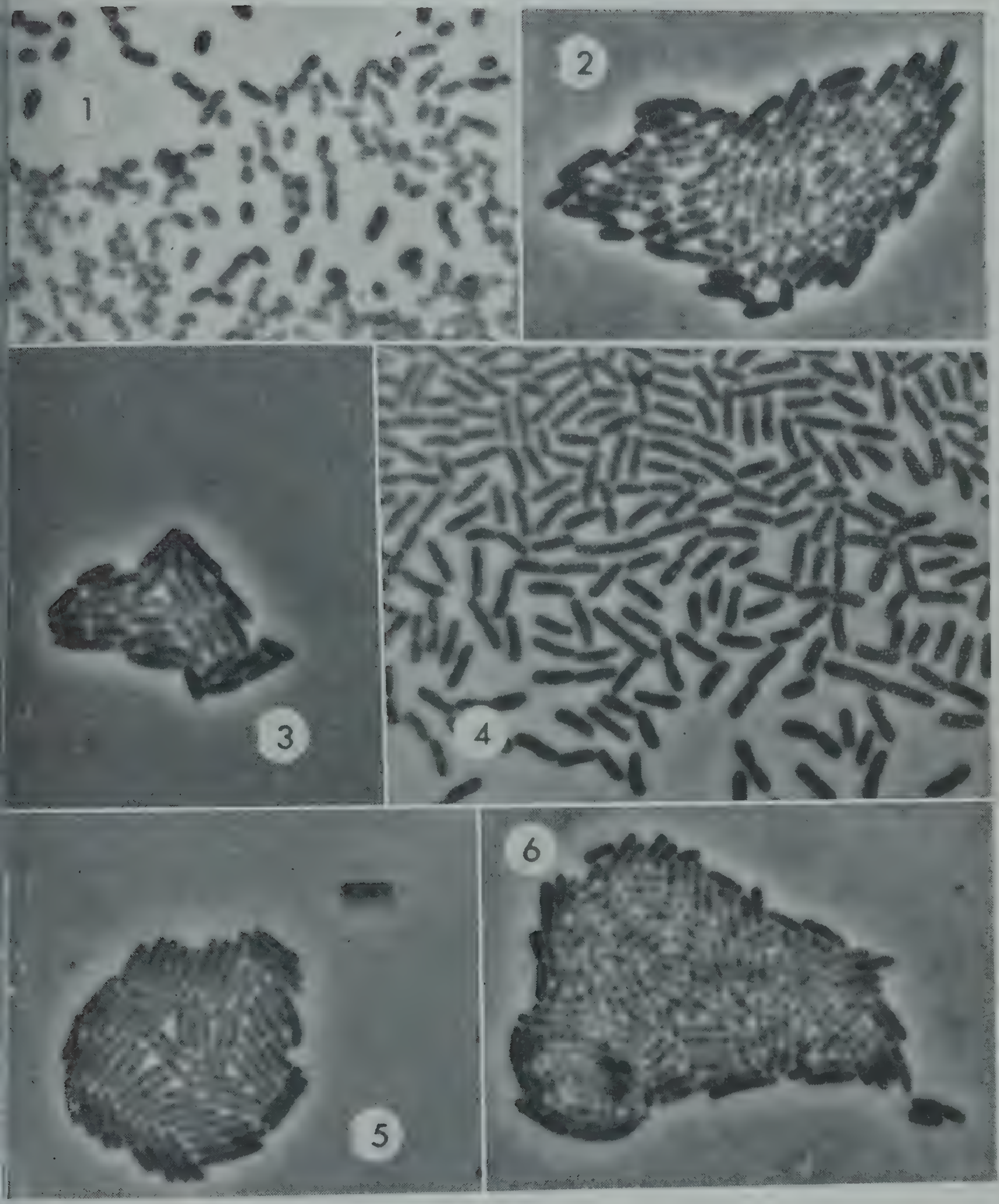


PLATE 14

. *Beneckea* sp. Slide culture. Original. × 1800. **2.** *Escherichia coli.* Slide culture. Orig-
. × 1800. **3.** *Aerobacter aerogenes.* Slide culture. Original. × 1800. **4.** *Klebsiella pneu-*
iae. Slide culture. Original. × 1800. **5.** *Paracolobactrum intermedium.* Slide culture.
inal. × 1800. **6.** *Erwinia carotovora.* Slide culture. Original. × 1800.

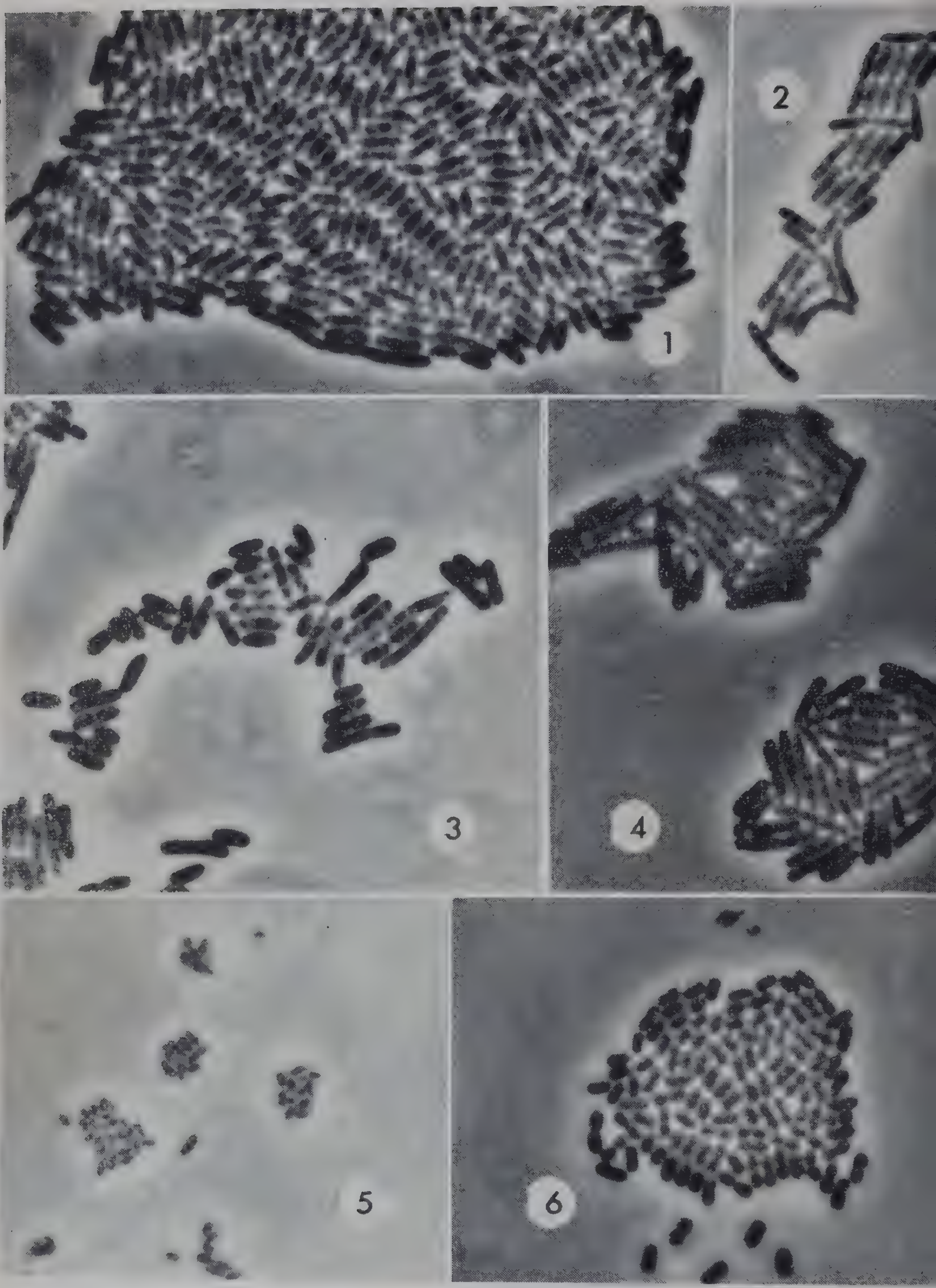


PLATE 15

1. *Serratia marcescens*. Slide culture. Original. × 1800. **2.** *Salmonella stanley*. Slid
ture. Original. × 1800. **3.** *Proteus morganii*. Slide culture. Original. × 1800. **4.** *Sh*
sonnei. Slide culture. Original. × 1800. **5.** *Pasteurella septica*. Slide culture. Origin
1800. **6.** *Bordetella pertussis*. Slide culture. Original. × 1800.

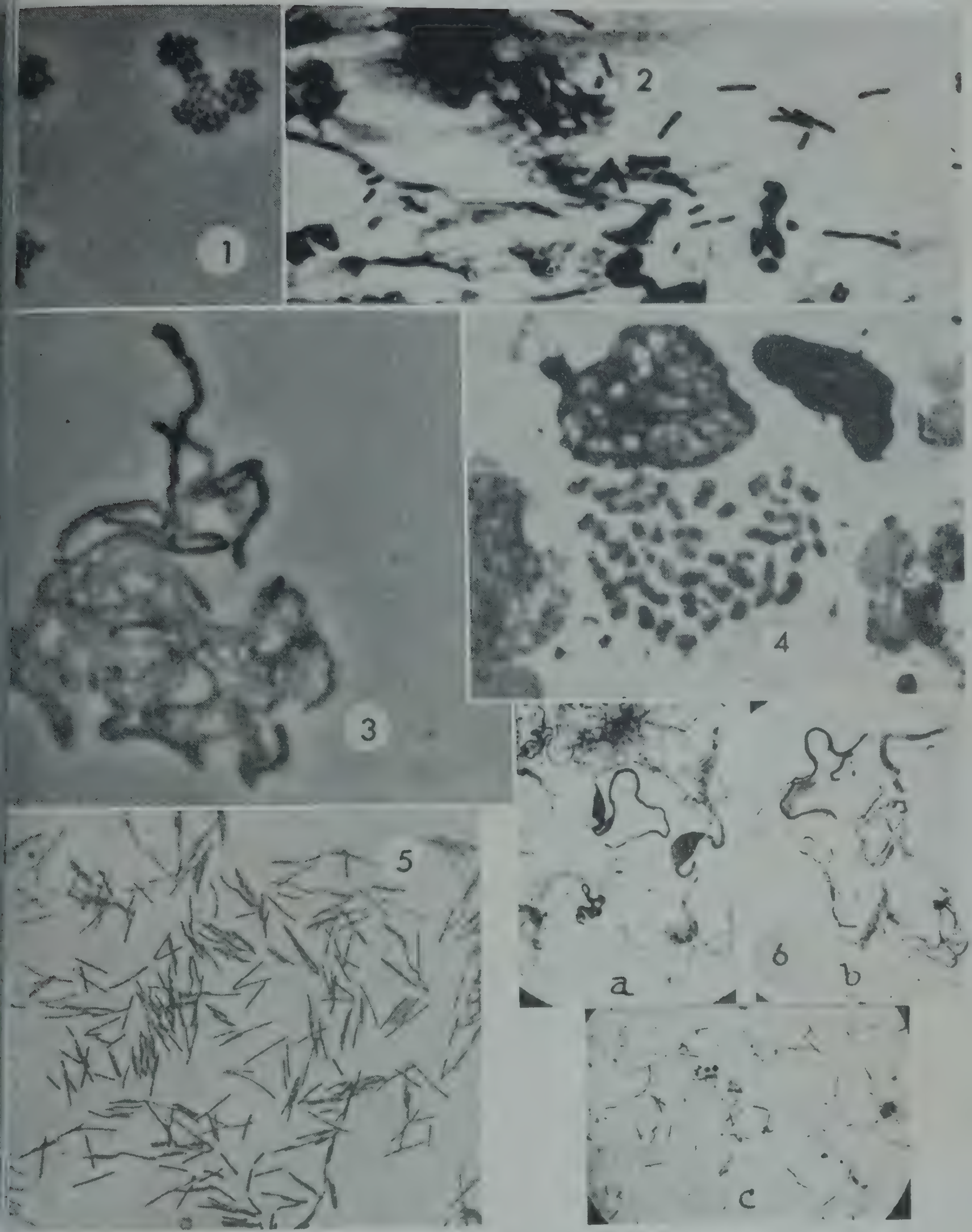


PLATE 16

1. *Brucella melitensis*. Slide culture. Original. × 1800. **2.** *Moraxella bovis*. Photomicro-
raph reproduced by permission from Jones and Little, J. Exptl. Med., *38*, 1923, 149. ×
00. **3.** *Haemophilus influenzae*. On Levinthal X- + V-factor agar. Slide culture. Original.
1800. **4.** *Calymmatobacterium granulomatis*. Photomicrograph reproduced by permission
om Anderson, De Monbreun, and Goodpasture, J. Exptl. Med., *81*, 1945, 25. × 1000. **5.** *Fu-*
bacterium fusiformis. Reproduced by permission from Varney, J. Bacteriol., *13*, 1927,
s × 500. **6.** *Sphaerophorus necrophorus*. Reproduced by permission from Dack, Dragstedt,
d Heinz, J. Infectious Diseases, *60*, 1937, 335. × 1000. *a* and *b*, 4-day anaerobic culture
strain 104 on cevitamic acid medium; *c*, 4-day anaerobic culture of strain 104 on blood
ar without cevitamic acid.

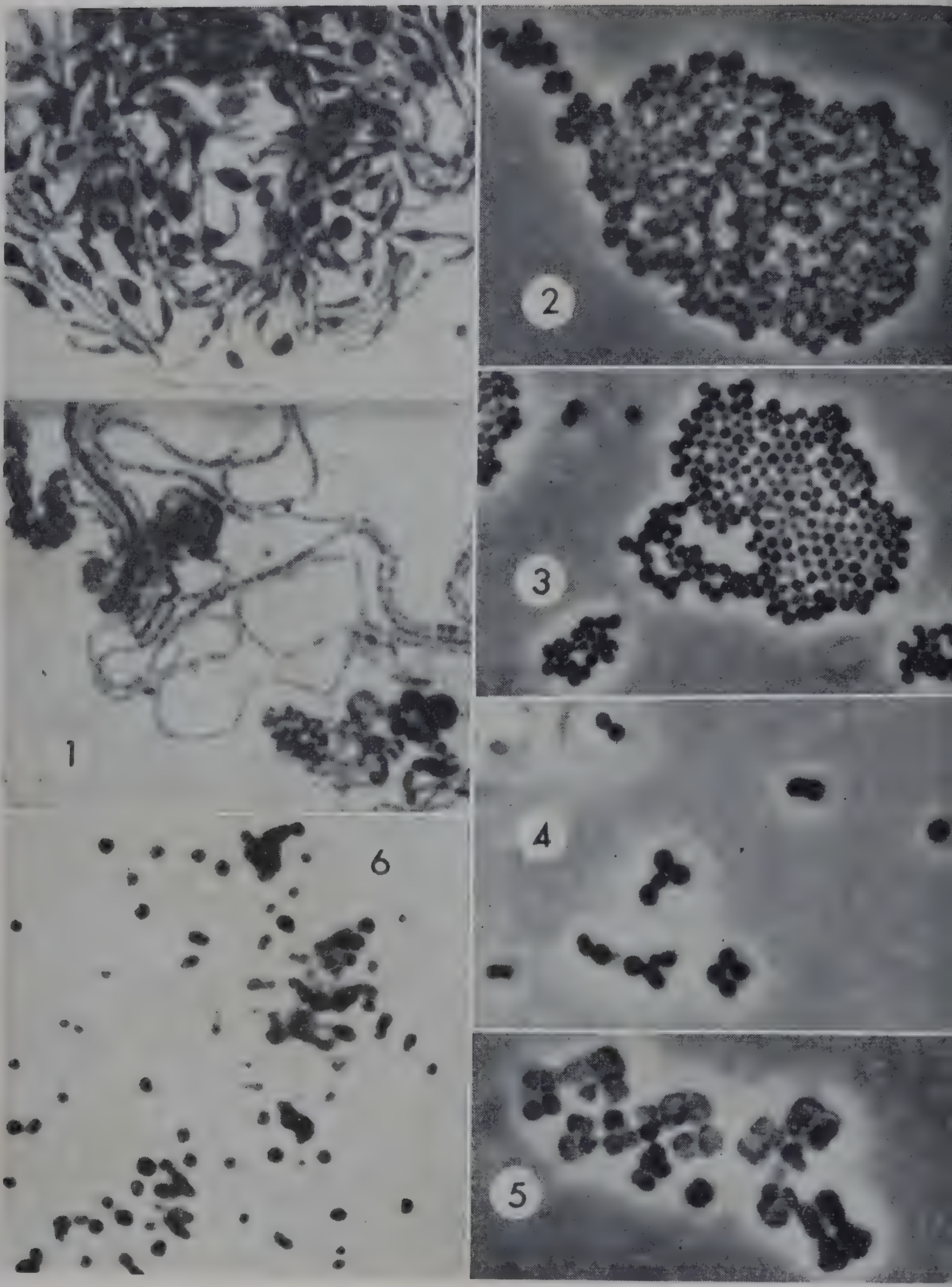


PLATE 17

1. *Streptobacillus moniliformis*. Photomicrograph reproduced by permission from D
J. Bacteriol., *44*, 1942, 64. × 2400. 2. *Micrococcus citreus*. Slide culture. Original. ×
3. *Staphylococcus aureus*. Slide culture. Original. × 1800. 4. *Gaffkya tetragena*. Slide cu
Original. × 1800. 5. *Sarcina lutea*. Slide culture. Original. × 1800. 6. *Methanococcu*
nielii. Photomicrograph reproduced by permission from Stadtman and Barker, J. Bact
62, 1951, 269. × 1420.

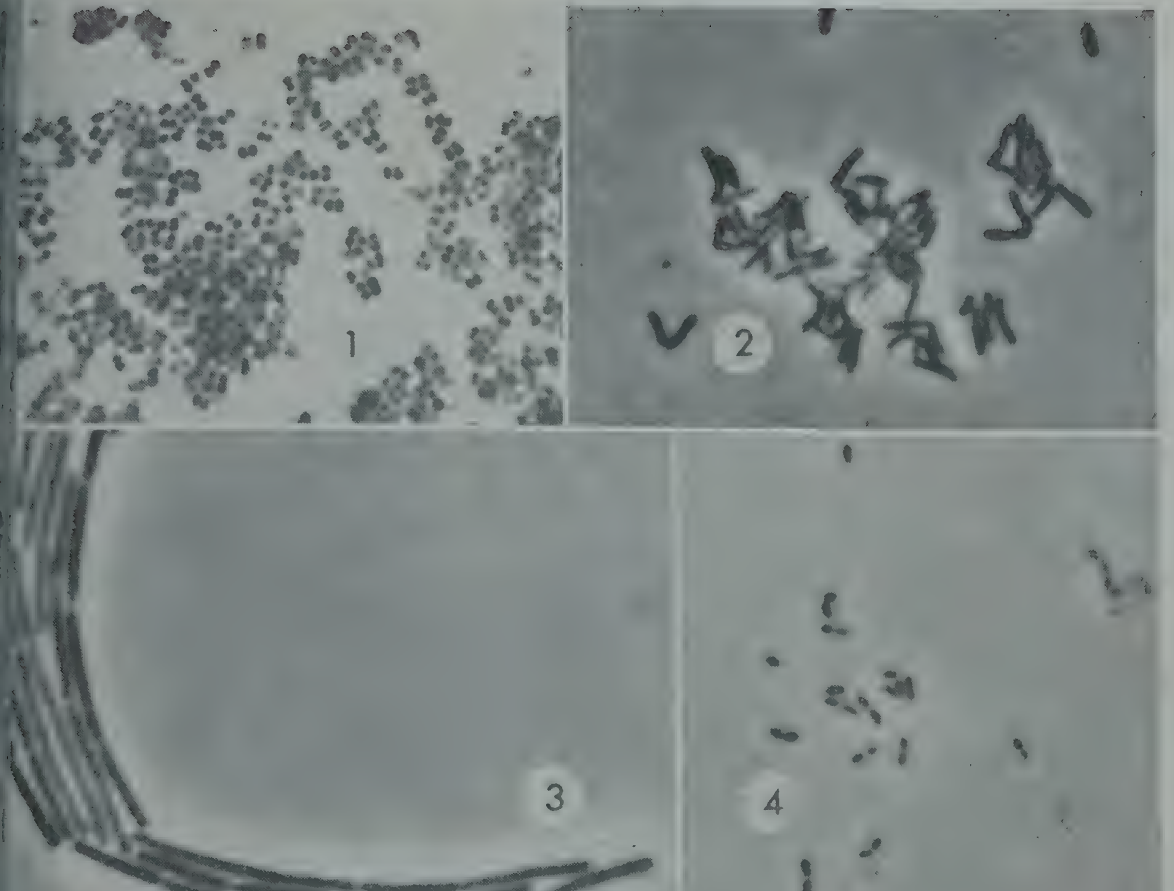


PLATE 18

1. *Neisseria catarrhalis*. Slide culture. Original. × 900. **2.** *Brevibacterium linens*. Slide
ture. Original. × 1800. **3.** *Kurthia zopfii*. Fringe of microcolony on slide culture. Orig-
l. × 1800. **4.** *Diplococcus pneumoniae*. Slide culture. Original. × 1800. **5.** *Streptococcus*
genes. Slide culture. Original. × 1800. **6.** *Pediococcus cerevisiae*. Slide culture. Original.
1800.

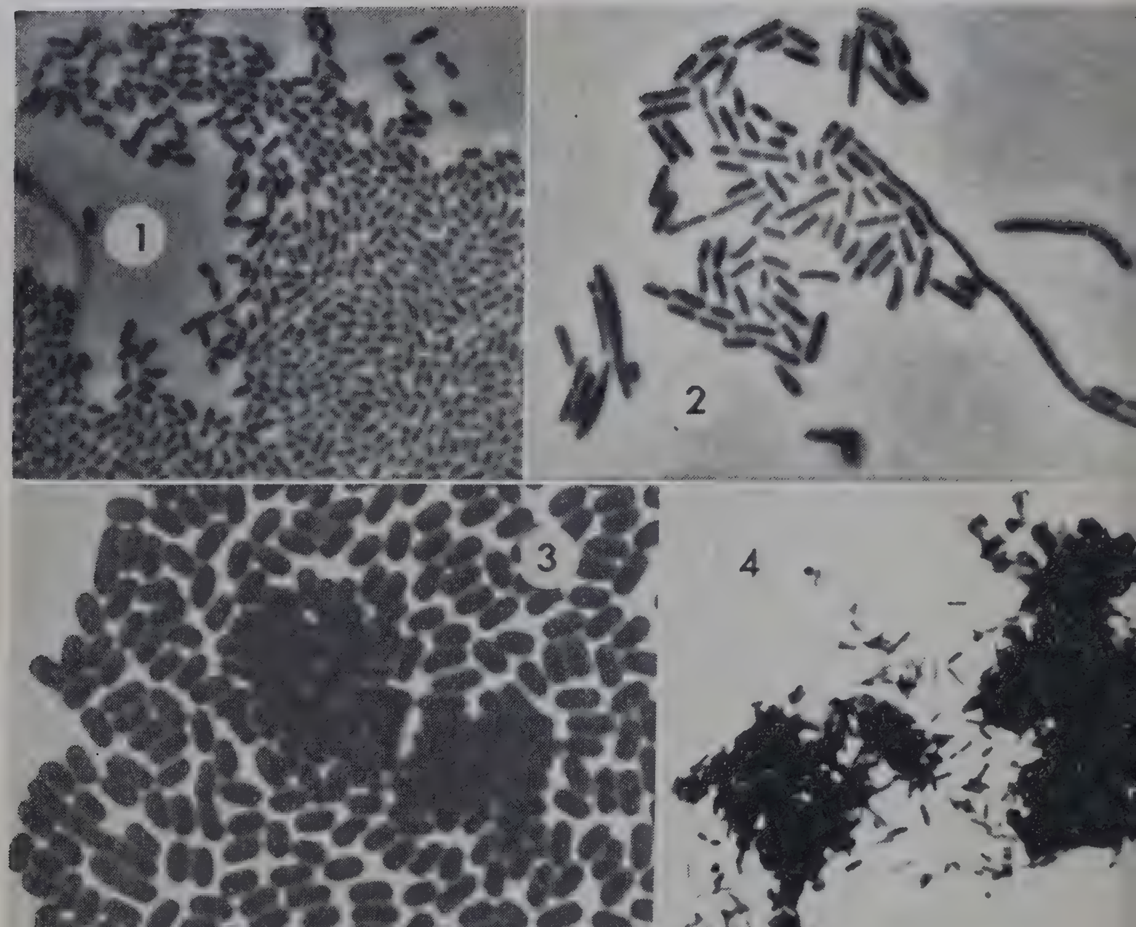


PLATE 19

1. *Leuconostoc mesenteroides*. Slide culture. Original. × 1800. 2. *Lactobacillus plant* Slide culture. Original. × 1800. 3. *Eubacterium* sp. Slide culture. Original. × 1800. 4. *bacterium* sp. Smear. Original. × 1800. 5. *Propionibacterium arabinosum*. Slide cu Original. × 1800. 6. *Butyribacterium rettgeri*. Reproduced by permission from Barke Haas. J. Bacteriol., *47*, 1944, 304. × 1100.

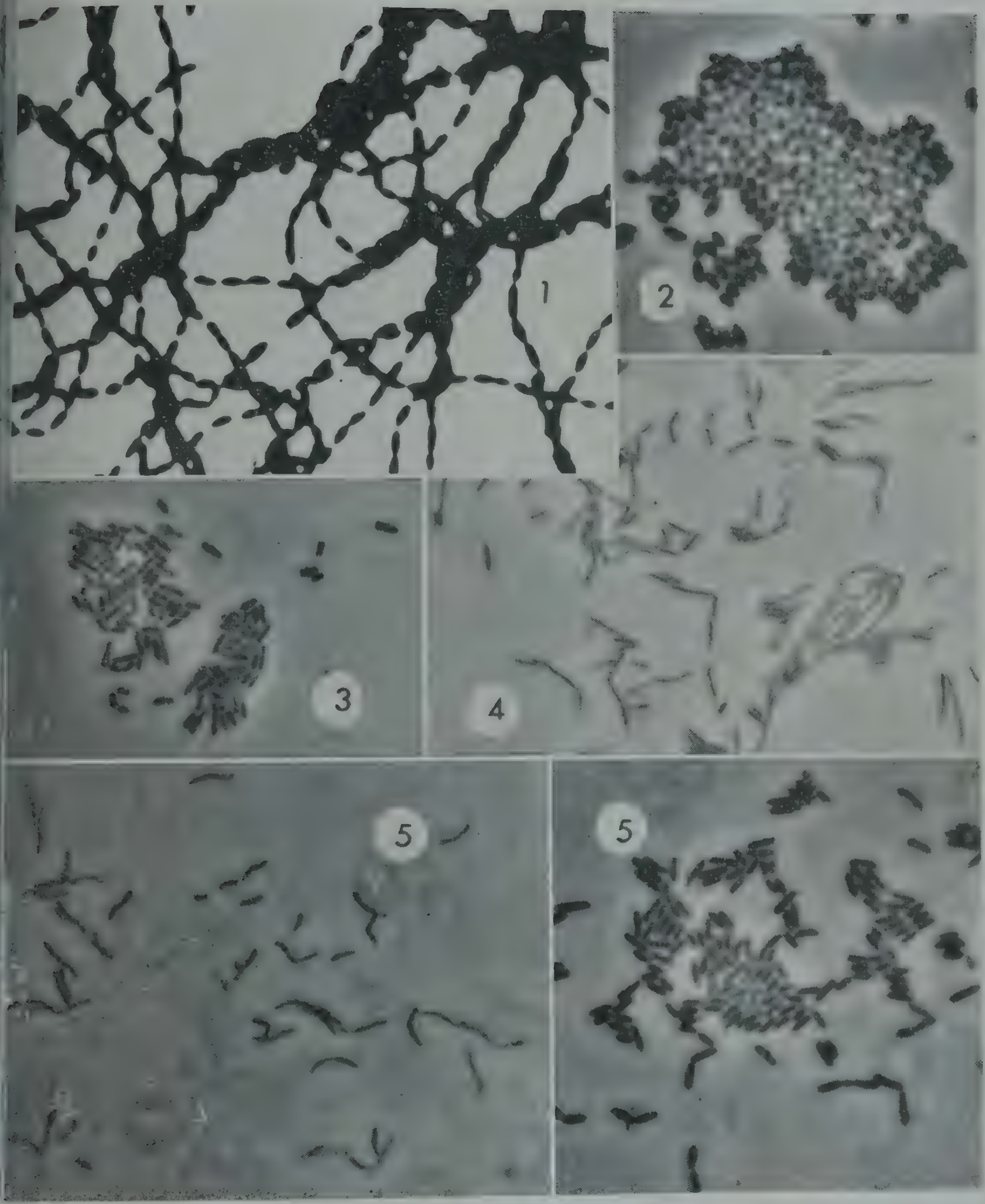


PLATE 20

. *Zymobacterium* sp. Courtesy of H. A. Barker, University of California. × 1500. **2.** *ynebacterium xerosis*. Slide culture. Original. × 1800. **3.** *Listeria monocytogenes*. Slide ure. Original. × 1800. **4.** *Erysipelothrix insidiosa*. Slide culture. Original. × 1800. **5.** *obacterium flavum*. Slide culture. Original. × 1800. **6.** *Cellulomonas biazotea*. Slide cul- . Original. × 1800.

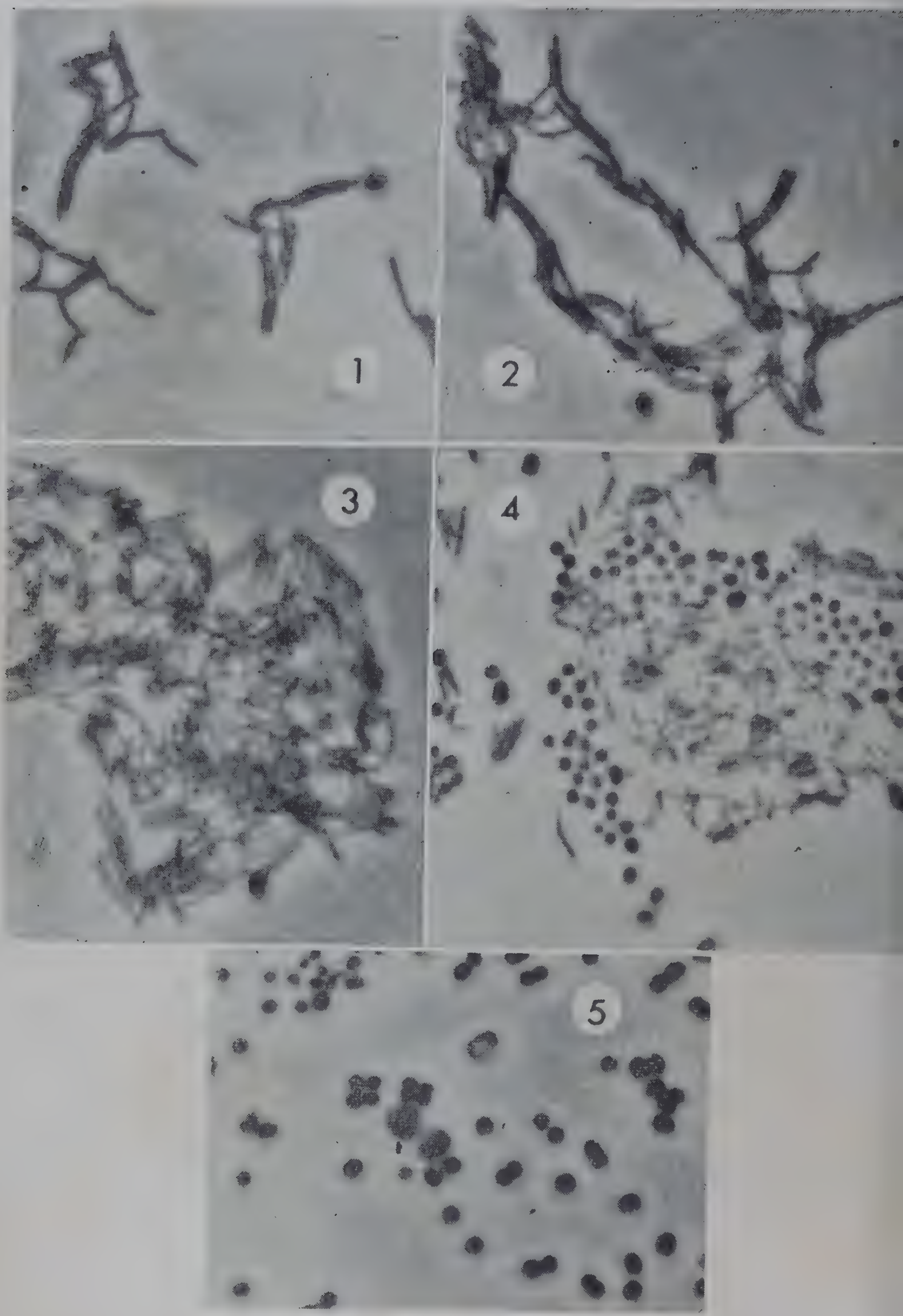


Plate 21

Arthrobacter sp. 1, 2, and 3, Development of microcolony. 4, Transference to co
Cocci and cystites. Original. × 1800.

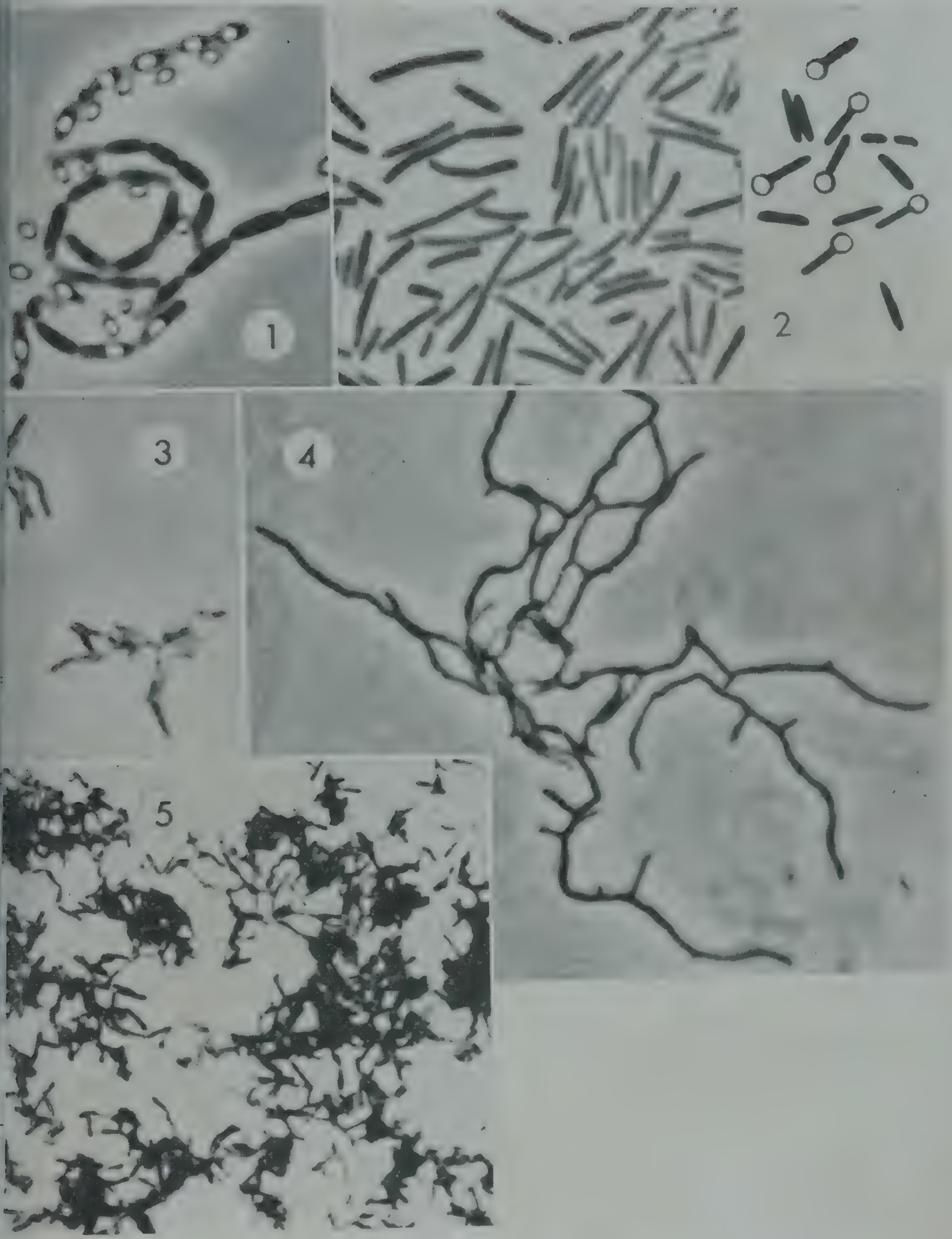


PLATE 22

Bacillus megaterium. Smear. Original. × 1800. 2. *Clostridium* sp. Slide culture and
drawing. Original. × 1800. 3. *Mycobacterium phlei*. Slide culture. Original. × 1800.
ocardia asteroides. Slide culture. Original. × 1800. 5. *Actinomyces*. Reproduced by per-
ion from Slack, J. Bacteriol., *43*, 1942, 197, fig. 1. × 1800.

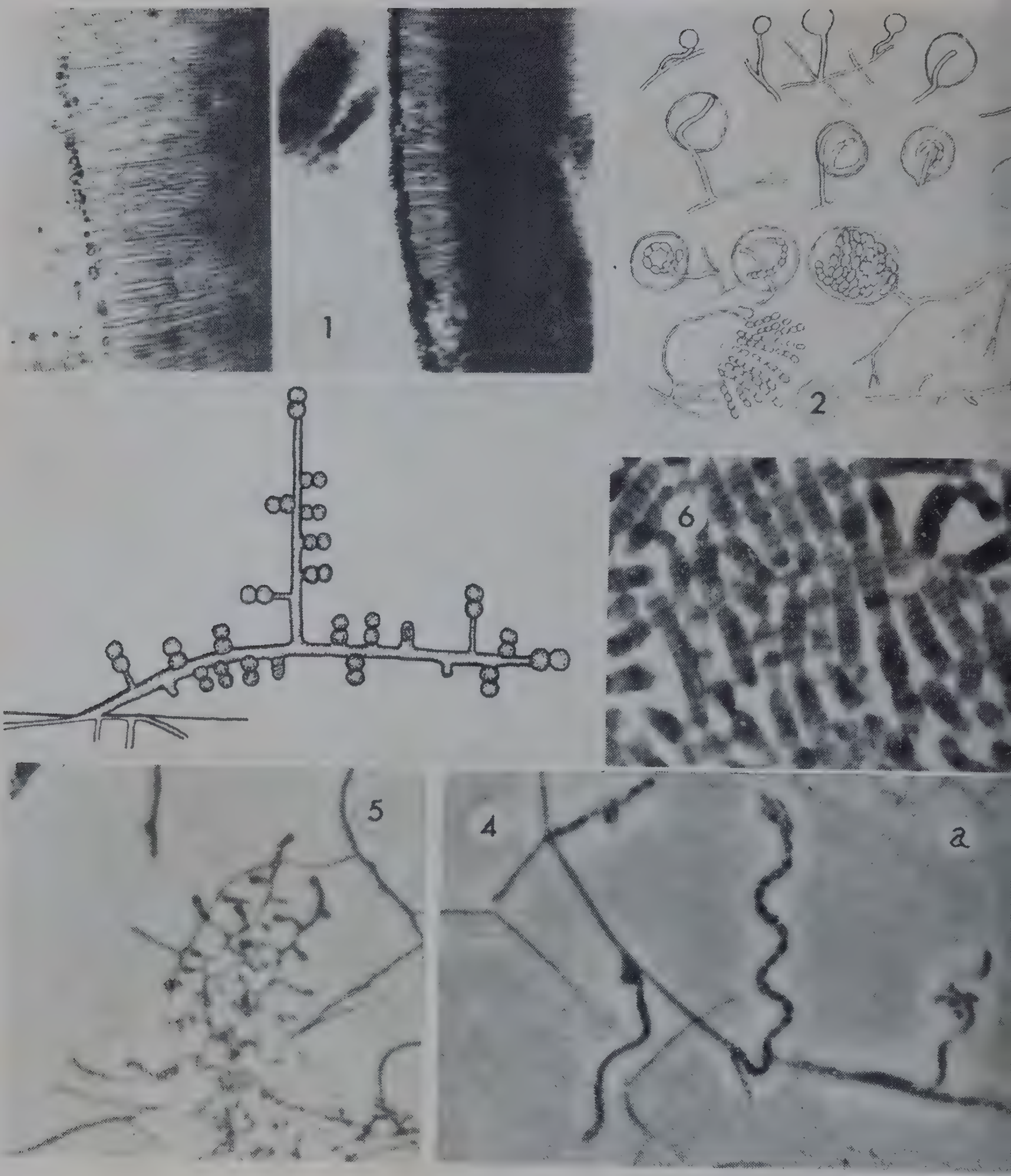


PLATE 23

1. *Actinoplanes philippinensis.* Photomicrograph reproduced by permission from
J. Elisha Mitchell Sci. Soc., *66*, 1950, 87. × 120. **2.** *Streptosporangium* sp. Reproduc
permission from Brummelen and Went, Antonie van Leeuwenhoek. J. Microbiol.
23, 1957, 385. × 820. **3.** *Waksmania* sp. Reproduced by permission from Le Cheval
Le Chevalier, J. Gen. Microbiol., *17*, 1957, 104. × 1700. **4.** *Streptomyces* sp. *a*, Slide
Original. × 1440. See next plate (24) for electron photomicrograph. **5.** *Micromonosp*
Slide culture. Original. × 1440. **6.** *Caryophanon latum.* Slide culture. Original. × 14

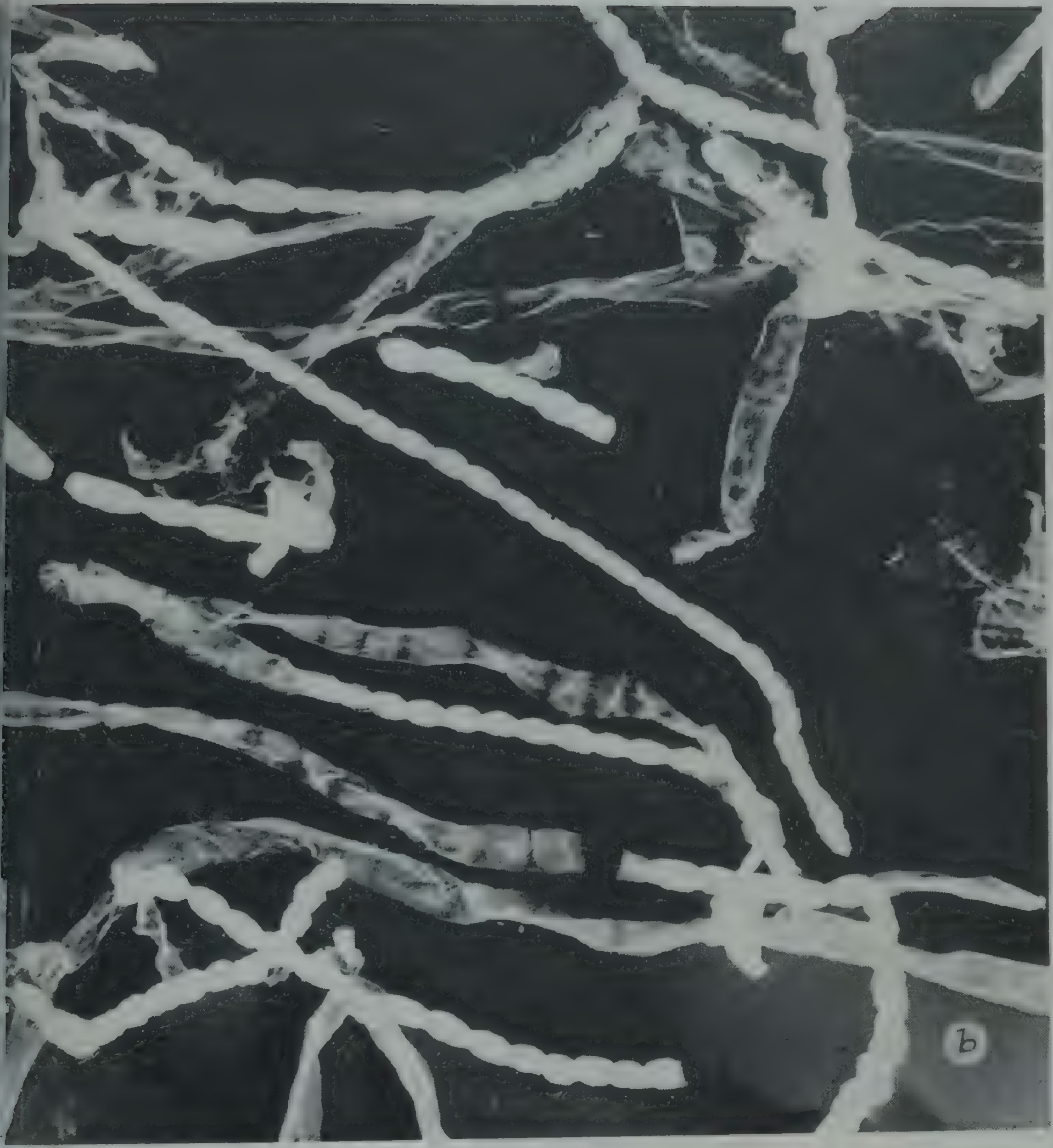


PLATE 24

reptomyces sp. Electron photomicrograph of chains of aerial conidia of *Streptomyces* ing clearly that these 'conidia' are formed intracellularly in the aerial hypha and not onstriction of the aerial hypha. Photograph by courtesy of T. R. Vernon, Plant Disease sion, D. S. I. R., Private Bag, Auckland.

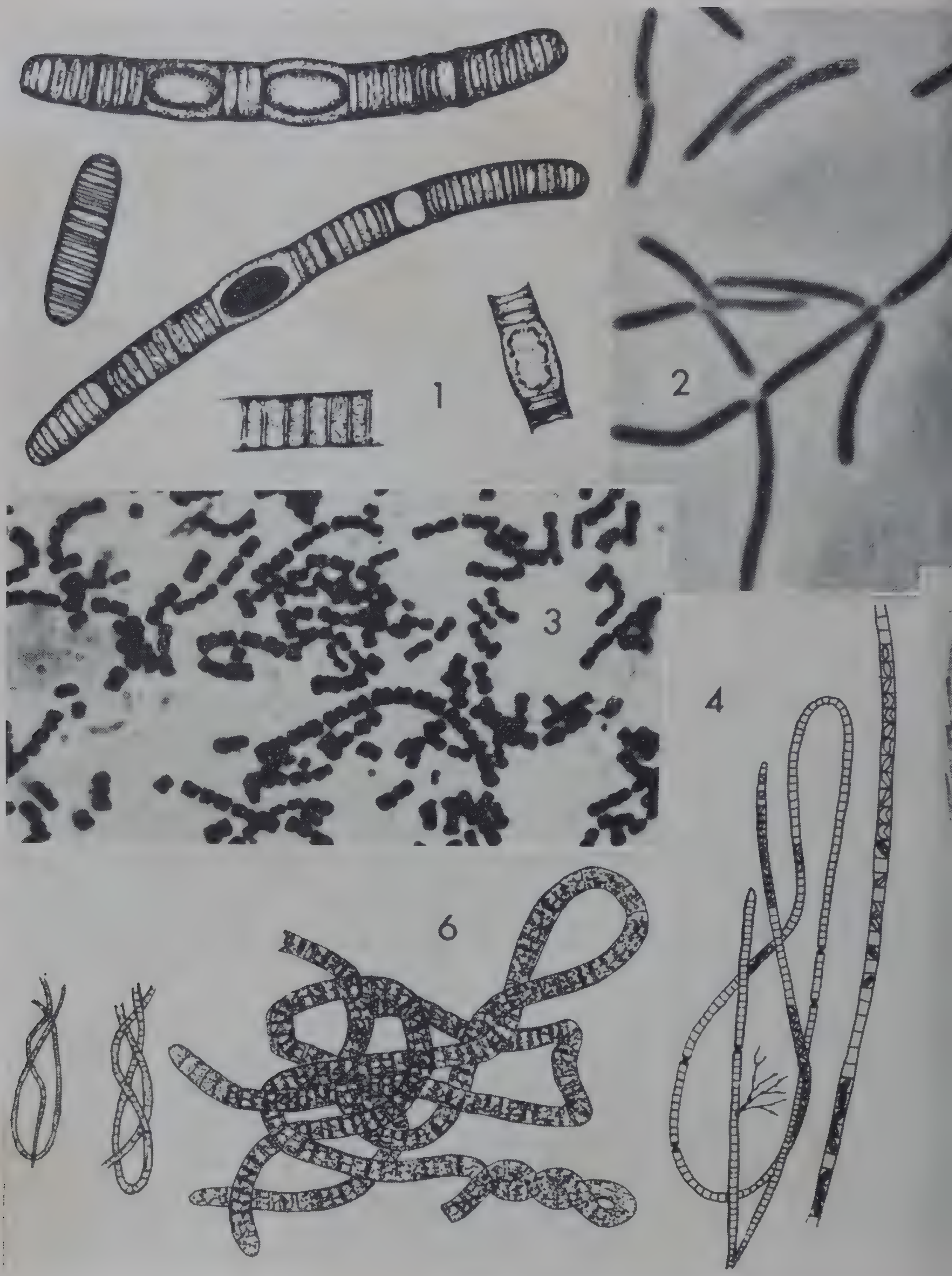


Plate 25

1. *Oscillospira guillermondii.* From Chatton and Pérard, Compt rend. soc. Biol.
74, 1913, 1159. Courtesy of Masson et Cie. × 1000. **2.** *Lineola* sp. Slide culture. O
× 900. **3.** *Simonsiella* sp. Smear. Original. × 900. **4.** *Arthromitus cristatus.* Rep
by permission from "Flora and Fauna within Living Animals" by J. Leidy in Smit
Inst. Publs. Contrib. to Knowledge, *5,* 1851. × 1850. **5.** *Coleomitus pruvotii.* Rep
by permission from Duboscq and Grassé, Arch. zool. exptl. et gén. *70,* 1930, Notes
vue, 28. × 1800. **6.** *Beggiatoa mirabilis.* From Cohn, Hedwigia, No. 6, 1865, facing
× 200.

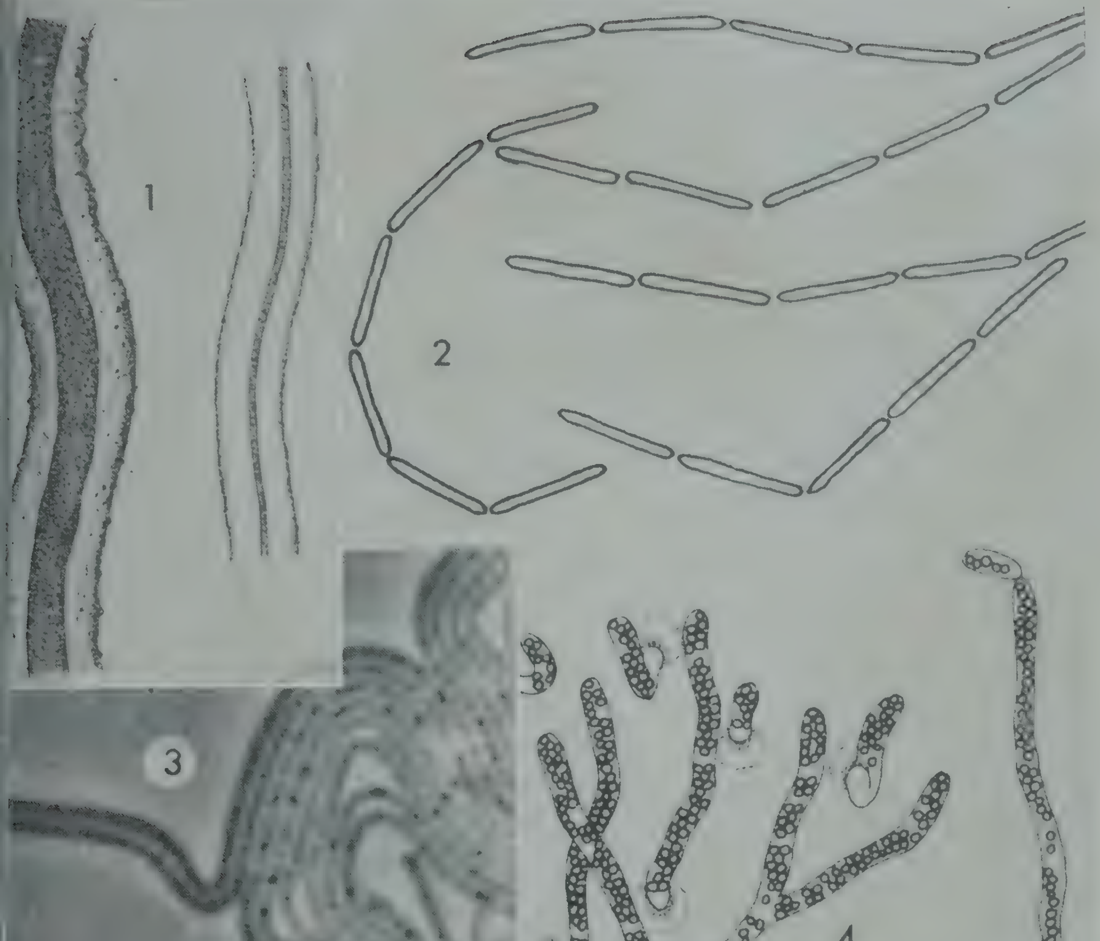
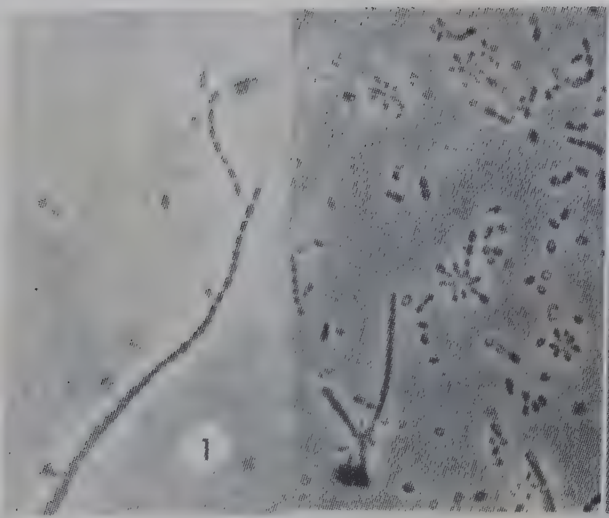
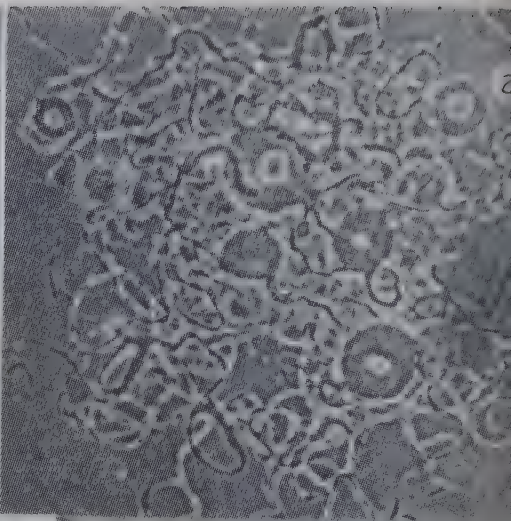
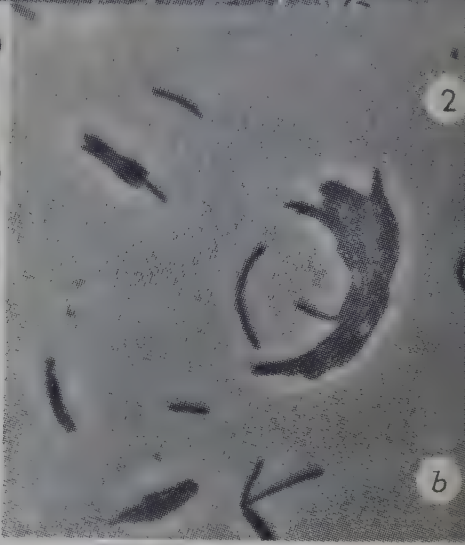


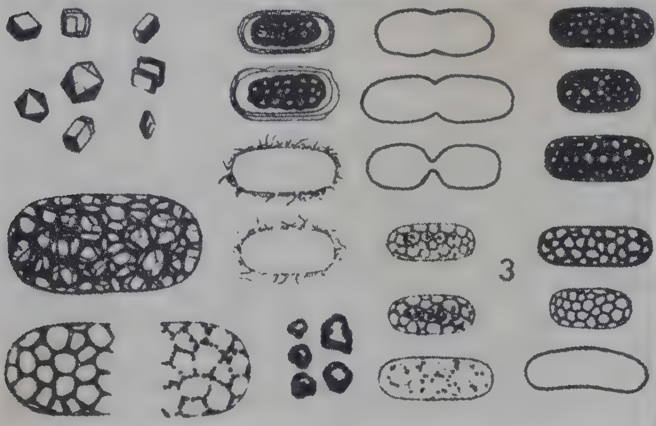
PLATE 26

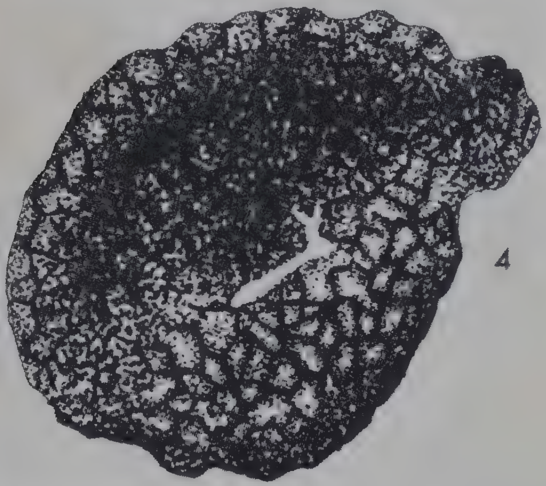
Thioploca schmidlei. From Lauterborn, Ber. deut. botan. Ges., *25*, 1907, 238. Courtesy
ustav Fischer, Stuttgart. × 120. **2.** *Bactoscilla mobilis*. Reproduced by permission from
gsheim, J. Gen. Microbiol., *5*, 1951, 124. × 1700. **3.** *Vitreoscilla* sp. Slide culture. Orig-
× 1530. **4.** *Thiothrix nivea*. Reproduced by permission from Winogradsky, Microbio-
du Sol, Masson et Cie, Paris. × 800. **5.** *Microscilla* sp. Slide culture. Original. × 1530.











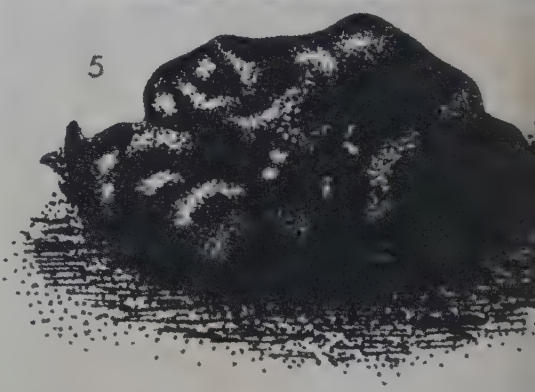
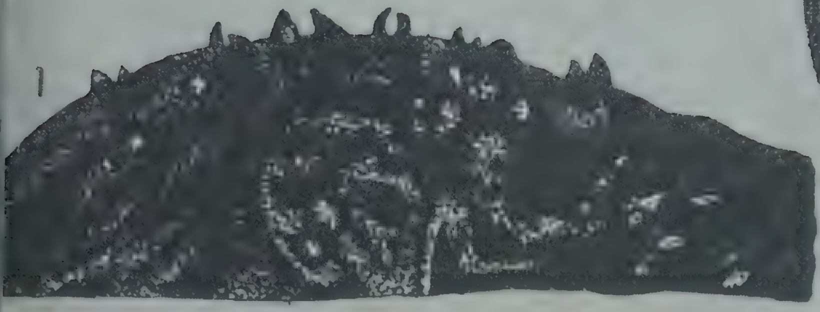
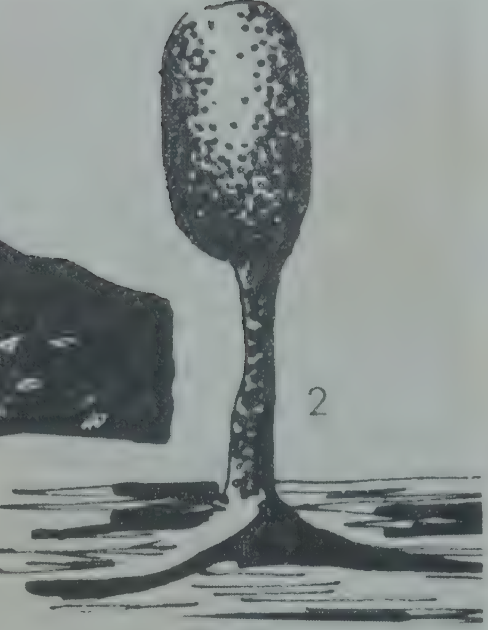


PLATE 27

1. *Leucothrix mucor*. Photomicrograph reproduced by permission from Harold an
ier, Bacteriol., Revs., *19*, 1955, 49. × 575. **2.** *Cytophaga* sp. *a*, Growth on agar show
gration of cells and slime trails. × 360. *b*, Cells migrating from the margin of the
Originals. × 1300. **3.** *Achromatium oxaliferum*. Reproduced by permission from W
Griffiths, Proc. Roy. Soc. London. B, *81*, 1909, Plate 9. × 360. **4.** *Sorangium sore*
From Quehl, Zentr. Bakteriol. Parasitenk., Abt. II, *16*, 1906, Plate I, fig. 2. Cour
Gustav Fischer, Stuttgart. × 210. **5.** *Archangium gephyra*. From Quehl, Zentr. Bal
Parasitenk., Abt. II, *16*, 1906, Plate I, fig. 7. Courtesy of Gustav Fischer, Stuttgart





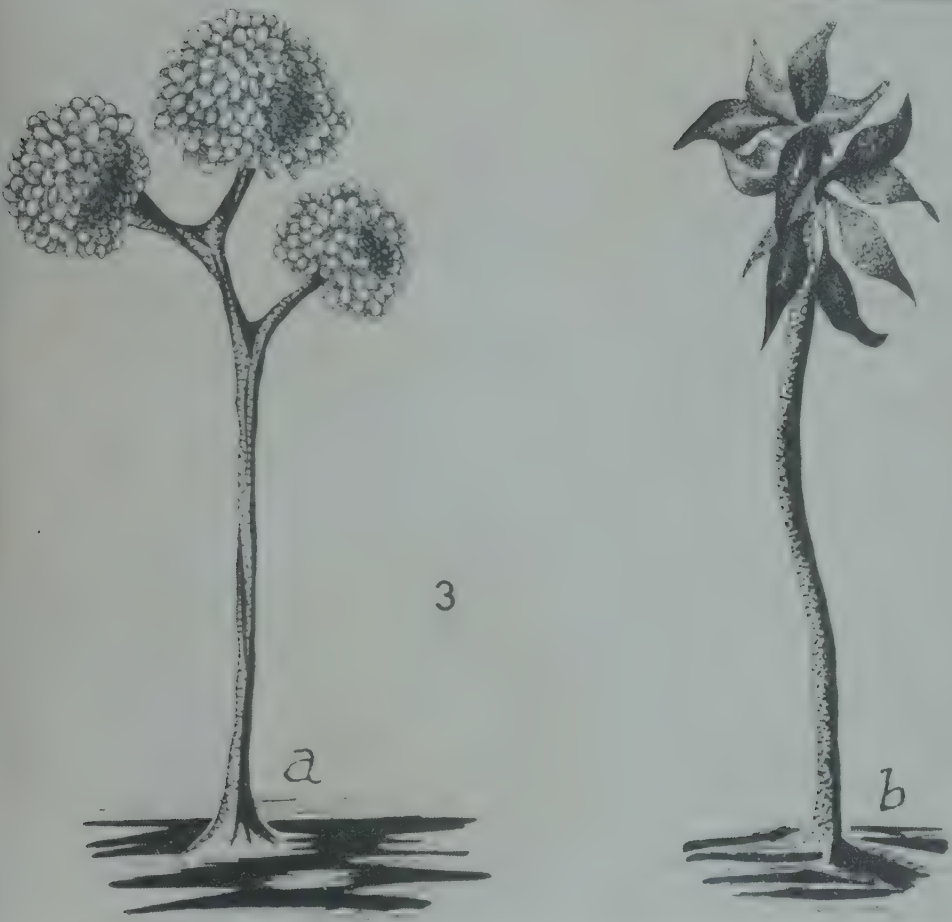


PLATE 28

Polyangium fuscum. From Quehl, Zentr. Bakteriol. Parasitenk., Abt. II, *16*, 1906,
e I, fig. 16. Courtesy of Gustav Fischer, Stuttgart. × 25. **2.** *Podangium gracilipes*.
n Quehl, Zentr. Bakteriol. Parasitenk., Abt. II, *16*, 1906, Plate I. Courtesy of Gustav
her, Stuttgart. × 700. **3.** *Chondromyces* sp. *a*, *C. crocatus* (Plate I, fig. 10) × 190. *b*, *C.*
ulatus (Plate I, fig. 13) × 220. From Quehl, Zentr. Bakteriol. Parasitenk., Abt. II, *16*,
. Courtesy of Gustav Fischer, Stuttgart.

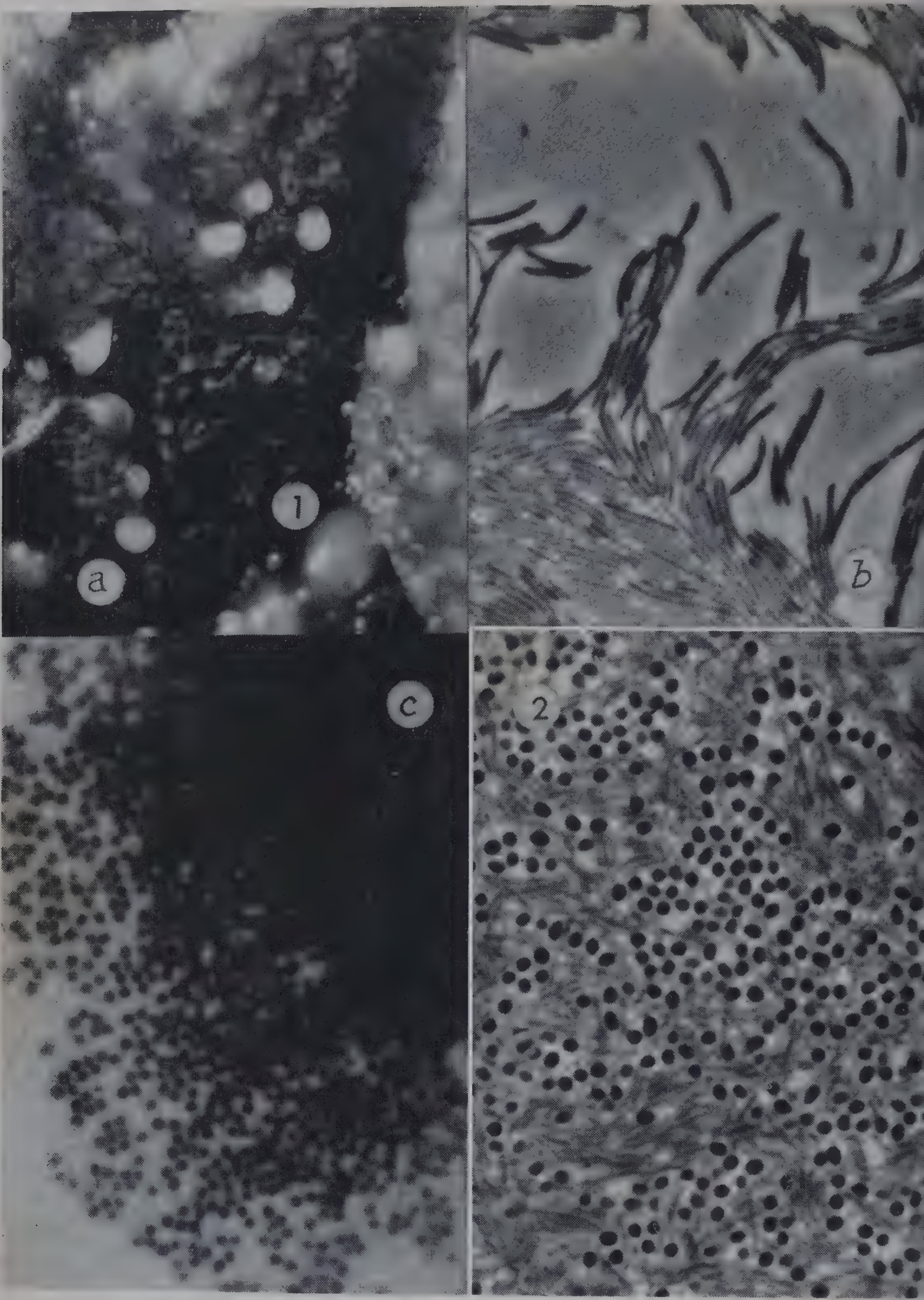
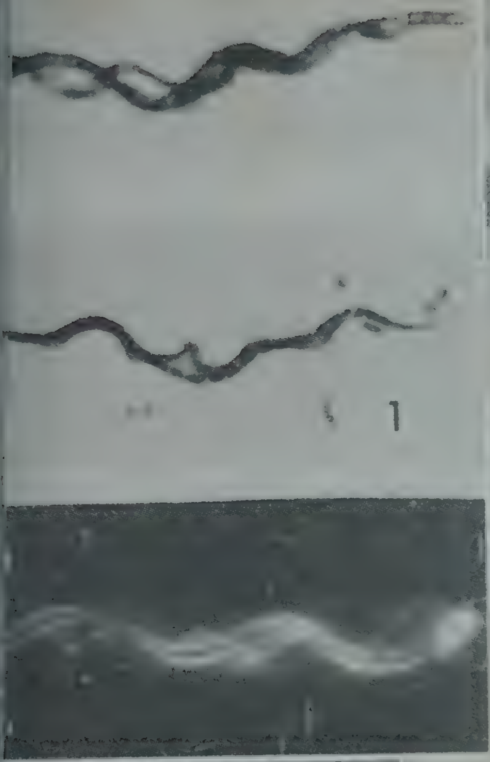
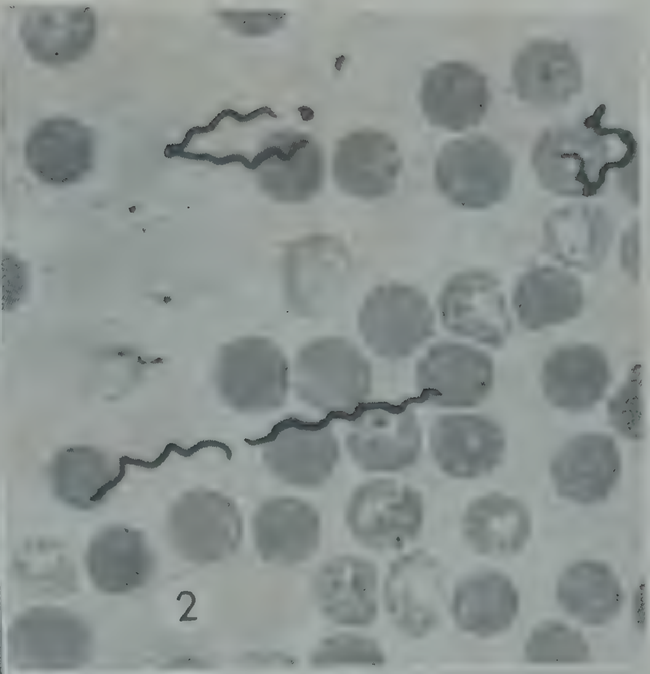
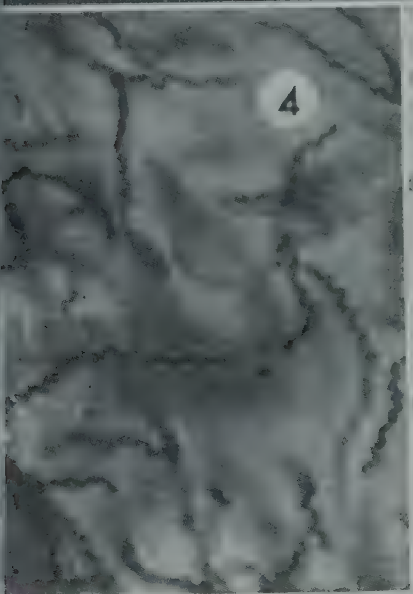


Plate 29

1. *Myxococcus* sp. Originals. *a*, Fruiting bodies on rabbit dung. × 2. *b*, Migrating from the margin of a colony. × 1530. *c*, Crushed fruiting body. × 1530. 2. *Sporocyto* sp. Microcysts among the flexible rods. Original. × 1530.







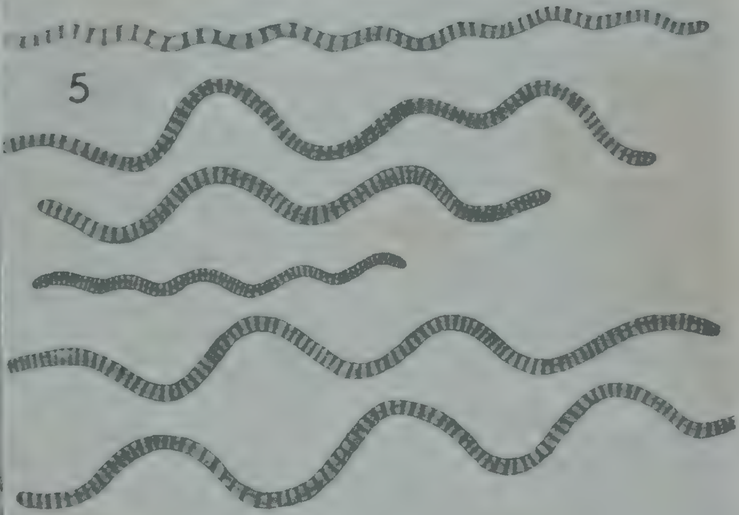


Plate 30

Cristispira sp. Reproduced by permission from Noguchi, J. Exptl. Med., *27*, 1918, e 29. *a*, *C. veneris*. × 1200. *b*, *C. balbianii*. × 1200. **2.** *Borrelia* sp. Blood smear. Original. 00. Prepared from a slide from Turtox Biological Supplies. 3. *Spirochaeta* sp. From ling, Arch. Protistenk. Bd. XXIII. Courtesy of Gustav Fischer, Stuttgart. × 750. **4.** *ponema pallidum*. Photomicrograph. × 2500. By courtesy of Turtox Biological Supplies. *aprospira grandis*. Reproduced by permission from Dimitroff, J. Bacteriol., *12*, 1926. × 1000.

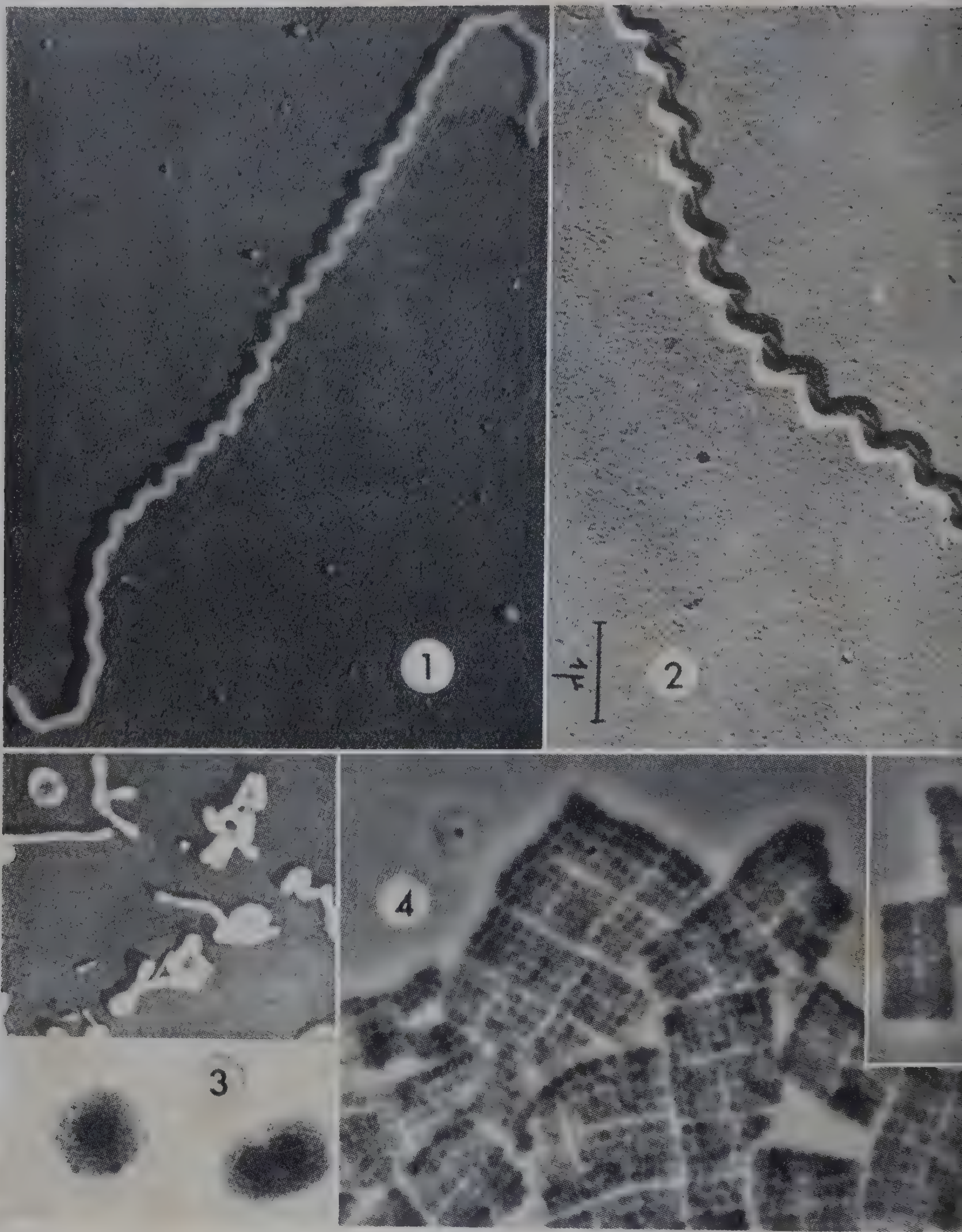


Plate 31

1. *Leptospira canicola*. Reproduced by permission from Gsell, Leptospirosen, Ha
ber, Bern, Switzerland. × 9000. 2. *Leptospira hebdomalis*. By courtesy of Dr. Elis
Mölbert. × 12,000. 3. *Mycoplasma* sp. Reproduced by permission from Klieneberger-
and Cuckow, J. Gen. Microbiol., *12*, 1955, 98, fig. 5. × 6600. 4. *Lampropedia hyalina*.
culture. Original. × 1800.

e and confined to the mycelium or e and diffusing into the medium. , yellow, orange, red and purple and brown pigments are common.

duction of conidia may fail entirely ne media and be prolific on others. s which maintain the permanent hing character of the mycelium (*cf.* *dia*), but which fail to produce co- under conditions of cultivation are l in a group of "sterile species."

atin is liquefied by 138/150. Ten s are negative, and two are not re- d.

rch is hydrolyzed by 119 species; it hydrolyzed by seven others. Twenty- species are not recorded.

rose is inverted by 13/25 species.

llulose is hydrolyzed by 24/51.

effler's inspissated serum is liquefied /19.

od is hemolyzed by 5/13 species.

mus milk is coagulated and peptonized eptonised without coagulation by 119 8 species tested. Since sugar fermenta- are rarely recorded it is not possible ate the true nature of the coagulum. possibly a soft rennet type clot.

itrite is produced from nitrate by 85 e 107 species tested.

rosinase is produced by only 5/23 es. (This test is of some importance he identification of species causing to scab disease.)

e conventional sugar reactions (*acid* *id and gas*) are recorded for only two es. In one of these discrimination is e between *acid production from* and *nilation* of carbohydrates. Since *Strepto-* s usually grow in the form of a sur- pellicle with no turbidity in liquids r main development is highly oxidative acid byproducts do not appear in unts sufficient to cause a change in the cator.

arbon assimilation tests have been re- ed for only nine species.

erobic.

ptimal temperature is dependent on habitat. Many have an optimum at 25° C. and others at 37°. Only four species, *S. thermophilus*, *S. thermofuscus*, *S. thermodiastaticus*, and *S. casei* are thermophilic; they grow at 50° to 65° C.

Streptomyces species are almost exclusively soil organisms. Eight species have been isolated from diseases in man. They are *S. listeri*, *S. galtieri* (septicemia in dogs), *S. upcottii* and *S. kimberi* (acholuric jaundice), *S. hortonensis* (parotid abscess), *S. beddardii* (splenic anemia), *S. somaliensis* (yellow grained mycetoma), *S. panjae* (abdominal ulcer) *S. willmorei* (streptothricosis of liver).

The following species have been isolated from potato scabs: *S. clavifer*, *S. fimbriatus*, *S. carnosus*, *S. craterifer*, *S. gracilis*, *S. praecox*, *S. setonii*, *S. praefecundus*, *S. viridogenes*, *S. loidensis*, *S. scabei* (principal species), *S. tenuis*, *S. marginatus*, and *S. sampsonii*.

S. ipomoeae and *S. poolensis* have been isolated from sweet potatoes and *S. tumuli* from mangels.

A large number of species produced antibiotics. It is largely due to this that so many ill-defined species are recorded. A separate list of names and antibiotics produced is presented in Table I.

Waksmania (Waks.ma′ ni.a) Lechevalier and Lechevalier, 1957. (J. Gen. Microbiol., *17*, 1957, 104.)

DIFFERENTIATING CHARACTERS: Free-living, filamentous, nonphotosynthetic, rod-shaped organisms, less than 2 μ wide, which in young cultures produce a branching mycelium that does not disintegrate as the culture ages. Conidia are produced in pairs, either sessile or on short (1 to 6 μ) conidiophores on the aerial mycelium or at the apex of an aerial hypha, with only one conidium of the pair attached to the mycelium. Sporangia are not produced; nonmotile; endospores not produced; *aerobic*; Gram-positive; heterotrophic.

Type species: *Waksmania roseum* Lechevalier and Lechevalier, 1957.

NOTES: This genus is not described in the

TABLE I

Antibiotics produced by species of Streptomyces

Name	Antibiotic produced	Name	Antibiotic pro
1. *S. albus*	Actinomycetin (some strains) Thiolutin (some strains) Endomycin (some strains)	36. *S. fimicarius*	Not mentio
2. *S. longisporus*	None	37. *S. felleus*	Picromycin
3. *S. globisporus*	None or weak	38. *S. achromogenes*	Achromovir
4. *S. anulatus*	Type not specified	39. *S. noursei*	Phalamycin nystatin
5. *S. rochei*	Borrelidin when grown on complex nitrogenous media	40. *S. roseochromogenes*	Roseomycin
6. *S. coelicolor*	Coelicolorin (some strains) and mycetin (some strains)	41. *S. cinnamonensis*	Identical wi tithiazic a thiozolidon
7. *S. pluricolor*	None	42. *S. olivochromogenes*	Type not sp
8. *S. cyaneus*	Weak	43. *S. resistomycificus*	Resistomycin
9. *S. vinaceus*	Vinactin	44. *S. diastatochromogenes*	Type not sp
10. *S. violaceus*	Type not specified	45. *S. flavochromogenes*	Not mentione
11. *S. verne*	Type not specified	46. *S. bikiniensis*	Streptomycin
12. *S. viridans*	None or (+) weak	47. *S. mirabilis*	Miramycin
13. *S. californicus*	Viomycin (some strains)	48. *S. antibioticus*	Actinomycin
14. *S. virgatus*	None	49. *S. griseocarneus*	Hydroxystrep mycin
15. *S. flaveolus*	Actinomycin (some strains)	50. *S. viridoflavus*	Candicidin-lik terial
16. *S. parvus*	Actinomycin	51. *S. globosus*	None
17. *S. xanthophaeus*	Type not specified	52. *S. cylindrosporus*	None
18. *S. cellulosae*	Type not specified	53. *S. viridochromogenes*	Type not spe
19. *S. rimosus*	Terramycin* and rimocidin	54. *S. purpureochromogenes*	Type not spe
20. *S. grisoflavus*	Type not specified	55. *S. phaeochromogenes*	Type not spe
21. *S. aureofaciens*	Chlortetracycline*	56. *S. aureus*	Fungicidin an teomycin (so strains)
22. *S. albidoflavus*	Type not specified	57. *S. tanashiensis*	Luteomycin
23. *S. lieskei*	None	58. *S. erythrochromogenes*	Type not spe
24. *S. flavovirens*	Type not specified	59. *S. collinus*	Not mentione
25. *S. celluloflavus*	Thiolutin	60. *S. lavendulae*	Streptothricin ehrlichin
26. *S. limosus*	Type not specified	61. *S. venezuelae*	Chloramphenic
27. *S. griseoluteus*	Griseolutein	62. *S. virginiae*	Actithiazic aci
28. *S. bobiliae*	Type not specified	63. *S. reticuli*	Neomycin or mycin like su
29. *S. aurantiacus*	Type not specified		
30. *S. griseolus*	None		
31. *S. fasciculus*	Type not specified		
32. *S. erythraeus*	Erythromycin*		
33. *S. flavogriseus*	Not mentioned		
34. *S. diastaticus*	Type not specified		
35. *S. canescens*	Ascosin		

* Of practical importance.

TABLE I.—(*Continued*)

Antibiotics produced by species of Streptomyces

Name	Antibiotic produced
S. netropsis	Netropsin
S. verticillatus	Weak
S. circulatus	Limited
S. rubrireticuli	Streptin (some strains)
S. flavus	Actinomycin (some strains)
S. ruber	Type not specified
S. niger	None
S. alboniger	Puromycin
S. abikoensis	Abikoviromycin
S. odorifer	Type not specified
S. roseoflavus	Flavomycin
S. putrificus	Not mentioned
S. citreus	None
S. fulvissimus	Not mentioned
S. chrysomallus	Actinomycin C (actinochrysin)
S. gougerotii	Type not specified
S. farinosus	None or very weak
S. albidus	Type not specified
S. cinnamoneus	Cinnamycin
S. violaceoniger	None
S. gedanensis	Not mentioned
S. griseus	Streptomycin (some strains), grisein (some strains), and candicidin (some strains)
S. longissimus	Weak
S. olivaceus	Type not specified
S. microflavus	Type not specified
S. cacaoi	Cacaomycetin
S. marinus	Not mentioned
S. novaecaesareae	None
S. exfoliatus	Type not specified
S. gelaticus	Type not specified
S. glaucus	Type not specified
S. rutgersensis	Limited
S. halstedii	Type not specified; magnamycin (some strains)
S. fumosus	None
S. lipmanii	Type not specified
S. hydroscopicus	Type not specified
S. longisporoflavus	Weak
S. candidus	Weak

Name	Antibiotic produced
102. *S. alboflavus*	Type not specified
103. *S. flocculus*	Not mentioned
104. *S. antimycoticus*	Helixin
105. *S. fradiae*	Neomycin* and fradicin
106. *S. albosporeus*	Type not specified
107. *S. purpurescens*	Rhodomycin
108. *S. longispororuber*	Type not specified
109. *S. oidiosporus*	None
110. *S. melanocyclus*	Type not specified
111. *S. acidophilus*	Type not specified
112. *S. rubescens*	Abikoviromycin
113. *S. viridis*	None
114. *S. thermophilus*	Thermomycin
115. *S. thermodiastaticus*	Not mentioned
116. *S. thermofuscus*	Not mentioned
117. *S. casei*	Not mentioned
118. *S. clavifer*	Not mentioned
119. *S. fimbriatus*	Not mentioned
120. *S. carnosus*	Not mentioned
121. *S. craterifer*	Not mentioned
122. *S. gracilis*	Not mentioned
123. *S. praecox*	Type not specified
124. *S. setonii*	Not mentioned
125. *S. praefecundus*	Not mentioned
126. *S. viridogenes*	Not mentioned
127. *S. loidensis*	Not mentioned
128. *S. wedmorensis*	Not mentioned
129. *S. scabies*	Type not specified
130. *S. tenuis*	Not mentioned
131. *S. marginatus*	Not mentioned
132. *S. salmonicolor*	Not mentioned
133. *S. maculatus*	Not mentioned
134. *S. coroniformis*	Not mentioned
135. *S. spiralis*	Not mentioned
136. *S. sampsonii*	Not mentioned
137. *S. intermedius*	Type not specified
138. *S. ipomoeae*	Type not specified
139. *S. poolensis*	Type not specified
140. *S. tumuli*	Not mentioned
141. *S. listeri*	Not mentioned
142. *S. galtieri*	Not mentioned
143. *S. upcottii*	Not mentioned
144. *S. hortonensis*	Not mentioned
145. *S. beddardii*	Not mentioned
146. *S. kimberi*	Not mentioned
147. *S. somaliensis*	Type not specified
148. *S. panjae*	Not mentioned
149. *S. willmorei*	Type not specified

Manual. Only one species is described. The vegetative mycelium is 0.3 to 1.2 μ in width. The paired conidia appear to arise by septation of the conidiophore followed by swelling of the two isolated cells. When mature, the conidia are easily separated. They are 1.5 to 2.0 μ in diameter, spherical to subglobose.

Growth occurs on a range of media both synthetic and complex. Excellent conidia formation occurs on rice, oatmeal, and mixed meal extract agars. The growth is a bright orange-pink.

Litmus milk is peptonized slowly without acid formation or coagulation.

Gelatin is slowly hydrolyzed.

Cellulose is slightly hydrolyzed.

Starch hydrolysis is variable according to the medium used.

No other information is given.

Aerobic.

Optimal temperature range, 25° to 35° C.

No antibiotics produced.

Found in soil.

Micromonospora (Mic.ro.mo.nos′po.ra) Ørskov, 1923. *M p. 822.* (Investigations into the Morphology of the Ray Fungi, Copenhagen, 1923, 147.)

DIFFERENTIATING CHARACTERS: Free-living, filamentous, nonphotosynthetic, rod-shaped organisms, less than 2 μ wide; produce, in young cultures, a branching mycelium which does not disintegrate completely as the culture ages. Conidia are produced singly on short side branches, not in sporangia; endospores not produced; Gram-positive; heterotrophic; nonmotile; no growth at 50° C.

Type species: *Micromonospora chalcea* (Foulerton) Ørskov.

NOTES: The genus is represented in the *Manual* by five species. All species have similar morphological characters. The short conidia-bearing hyphae appear in clusters towards the center of the developing colonies. Branching of the conidiophores occurs but each branch bears only one conidium. Conidia stain very intensely when first formed but may develop a hollow appearance as they age. Th
ellipsoidal or spherical.

According to Bisset and Moore (
Microbiol., *3*, 1949, 387) individual
the mycelium are unicellular and
without producing a septum at th
where the branch leaves the mother

Information on nutritional requir
in the *Manual* is lacking. Growth oc
glucose-asparagine agar and othe
thetic media. Colonies are opaque,
or rugose, and usually pigmented
yellow, orange, or green. The pigm
not soluble. Obvious aerial mycel
usually lacking. It is limited to
conidiophores. The spore layer m
dark brown to black. Colonies are
to emulsify.

Iron is precipitated from ferri
monium citrate by some species (a
note).

Gelatin is liquefied by all species.

Litmus milk is peptonized by 4/4

Starch is hydrolyzed by all specie

Cellulose is hydrolyzed by 2/5.

Sucrose is inverted by 3/5.

No information has been given
reactions on other carbohydrates.

Nitrites are produced by 3/5 spec

Aerobic or *anaerobic.**

Optimal temperature is 35° to 37°

All species have been isolated fro
soil.

Thermoactinomyces (Ther.mo.
no.my′ces) Tsiklinsky, 1899. *M p*
(Ann. inst. Pasteur, *13*, 1899, 501; a
1903, 206.)

DIFFERENTIATING CHARACTERS:
living, filamentous, nonphotosyn
rod-shaped organisms, less than 2 μ
produce, in young cultures, a bra
mycelium which does not disintegr
the culture ages; conidia are pro
singly on short conidiophores which
be simple or branched; sporangia no

---

* Hungate (J. Bacteriol., *51*, 194
describes an anaerobic cellulose-dig
organism belonging to this genus.

no endospores produced; Gram-
e; heterotrophic; will grow at 50° C.
e species: *Thermoactinomyces vul-*
Tsiklinsky.

es: There are only three listed
. The morphology is similar in all
Growth occurs readily on synthetic
trient agar. Colonies prior to sporula-
re colorless or yellowish. They be-
powdery on sporulation, the conidial
being white or grayish green. A
e wine-colored pigment is produced
cose-yeast agar by one species.

tin is liquefied by all species.

nus milk is coagulated and peptonized
3.

rch is hydrolyzed by 2/3 species.

information is given on the reac-
on carbohydrates.

obic.

timal temperature. All species will
at 50° C; two of the three will grow
.

lated from high temperature com-
.

tinoplanes (Ac.ti.no.pla′ nes)
h, 1950. *M p. 826.* (J. Elisha Mitchell
oc., *66*, 1950, 87.)

FERENTIATING CHARACTERS: Free-
, filamentous, nonphotosynthetic or-
ms, less than 2 μ in width, which
ce, in young cultures, a branching
lium that does not disintegrate as the
re ages; conidia, 1 to 1.5 μ in diameter,
roduced in coiled chains or irregularly
n sporangia. The sporangia are 8.4 to
in diameter, borne on unbranched
l hyphae; conidia are *motile* and
inate to produce the branched my-
m which penetrates into submerged
t tissue; no endospores produced;
bic; Gram-positive; heterotrophic.

ype species: *Actinoplanes philippinensis*
ch, 1950.

TES: Only one species is listed.

reptosporangium (Strep.to.spo.
gi.um) Couch, 1955. *M p. 828.* (J.
ha Mitchell Sci. Soc., *71*, 1955, 148.)

DIFFERENTIATING CHARACTERS: Free-living, filamentous, nonphotosynthetic organisms which produce, in young cultures, a branching mycelium that does not disintegrate as the culture ages. Spherical, nonmotile conidia, 1.8 to 2.0 μ in diameter, are produced in coils within sporangia. The sporangia are 7 to 19 μ in diameter and are produced apically on simple or branched aerial hyphae. Conidia are forcibly ejected from a protuberance which forms on the sporangia when the latter are immersed in water. Growth over the surface of submerged plant tissue; no endospores produced; *aerobic*; Gram-positive; heterotrophic.

Type species: *Streptosporangium roseum* Couch.

NOTES: Only one species is listed.

Caryophanon (Ca.ry.o′ pha.non) Peshkoff, 1940. *M p. 831.* (J. Gen. Biol. (U.S.S.R.), *1*, 1940, 611.)

DIFFERENTIATING CHARACTERS: Colorless, uniseriately multicellular, rod-shaped microorganisms which may exceed 2 μ in diameter; do not produce endospores; motile with peritrichous flagella; *aerobic*; Gram-negative.

Type species: *Caryophanon latum* Peshkoff.

NOTES: Only two species, *C. latum* and *C. tenue*, are listed. The former reaches 3 μ in width and the latter 1.5 μ. Individual rods are composed of a series of cells much wider than long and are terminated at both ends with hemispherical cells. Rods may be joined to form long chains. Individual cells within the rod may separate as flat cylindrical units. These may predominate at pH 7.0. Uneven development of cells within the rods may result in some grotesque shapes.

Nuclear material can be demonstrated by special staining methods.

Both species grow well on peptone-yeast extract-acetate agar and on dung infusion agar at pH 7 to 8. Rough colonies predominate in old cultures.

No information is given on biochemical and other properties.

Found in fresh cow dung.

Lineola (Li.ne.o′ la) Pringsheim, 1950. *M p. 832.* (J. Gen. Microbiol., *4*, 1950, 198.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, colorless, nonsporing, rod-shaped organisms, less than 2 μ wide and 10 to 15 μ long; motile with peritrichous flagella; arranged in chains usually over 100 μ long. Individual rods are characterized by constrictions which later develop into cross walls followed by division. Cells may branch, the branching appearing at or near the site of constriction but not visibly connected to the main axis. Form small, bluish white iridescent colonies on peptone-yeast extract agar, particularly with added acetate. Larger spreading fimbriate colonies are also produced in a patch-like fashion.

Type species: *Lineola longa* Pringsheim.

NOTES: Two species are described. No other information is available.

Simonsiella (Si.mon.si.el′ la) Schmid, 1922. *M p. 833.* (Zentr. Bakteriol. Parasitenk., Abt. I, Orig., *88*, 1922, 504.)

DIFFERENTIATING CHARACTERS: Colorless disc-like cells arranged in chains with the flat sides adjacent. Gram-positive (author's note); nonmotile.

Type species: *Simonsiella muelleri* Schmid, 1922.

NOTES: The *Manual* gives only references to the morphology of the cells. The cells of *Simonsiella muelleri* measure 0.4 to 0.7 μ by 2.0 to 3.0 μ and are arranged side by side in a chain with the long axis of each cell at right angles to the axis of the chain. The cells occur characteristically in pairs or groups of four. In short chains the end cells are rounded.

Three species have been listed.

Aerobic.

Occur in oral cavities of man and animals.

Oscillospira (Os.cil.lo.spi′ ra) Chatton and Pérard, 1913. *M p. 834.* (Compt. rend., *65*, 1913, 1159.)

DIFFERENTIATING CHARACTERS: ing, multicellular, colorless organism than 2 μ in width; motile with perit flagella; produce a large endospore ently by fusion with several cells the trichome; occur in large num the cecum of the guinea pig.

Type species: *Oscillospira guillie* Chatton and Pérard.

NOTES: There is only a single spe is a colorless, multicellular, rod-sha ganism, straight or slightly curv proximately 5 μ wide and up to 100 Individual cells are 1 to 2 μ long within the rod are usually squar whereas terminal cells are hemisp Separation of long rods into shorte is preceded by the formation of a bi disc similar in appearance to that by *Oscillatoria* in the formation of gonia. Occasionally large oval spo produced at some point in the rod.

The organism is peritrichously flag but is rarely seen to be motile in scopic preparations.

It has never been isolated so infor on its nutritional and biochemical ties is lacking.

Found in the cecum of the guinea

Arthromitus (Ar.thro.mi′ tus) 1849. *M p. 835.* (Proc. Acad. Na Phila., *4*, 1849, 227.)

DIFFERENTIATING CHARACTERS: less, uniseriately multicellular, rod- microorganisms which develop from fast attached to the wall of the alim canal of millipedes, toads, and insects motile; endospores are produced in in ual cells and are obliquely situated.

Type species: *Arthromitus cr* Leidy.

NOTES: There are four listed specie individual cells in the trichomes are than wide and vary in width from 5.0 μ, depending on the species. Tric may be over 2000 μ long.

Morphological data only are give

Coleomitus (Co.le.o.mi′ tus) Du

rasse, 1930. *M p. 836.* (Arch. zool. t gén., *70,* 1930, Notes et Rev., 28.)

ERENTIATING CHARACTERS: Color- uniseriately multicellular organism hyaline sheath; 0.3 μ wide and up μ long. Individual bacillary elements to 4 μ long. Endospores 0.8 to 0.9 μ to 2.0 μ, each containing an eccen- y placed volutin granule, are pro- .

e species: *Coleomitus pruvotii* Du- and Grasse.

ES: Only one species is described. scribed from the intestines of ter- .

ggiatoa (Beg.gi.a.to′a) Trevisan, *M p. 838.* (Prospetto della Flora nea, 1842, 56.)

FERENTIATING CHARACTERS: Free- , colorless, uniseriately multicellular, le organisms; motile without flagella; growing in the presence of hydrogen le, they deposit sulfur inside the cells.

pe species: *Beggiatoa alba* (Vaucher) isan.

TES: Six species are listed. They are entiated solely on the width of the which ranges from less than 1 μ to than 70 μ. The validity of the differ- tion is questionable.

ce thought to be strictly autotrophic dependent on H_2S for their metabo- they have been shown to be hetero- ic and not dependent on H_2S. Their ion is difficult, and until they can be ssfully maintained in pure culture for ded periods and subjected to further , some characteristics must remain in t.

und in stagnant waters, both fresh salt.

hiospirillopsis (Thi.o.spi.ril.lop′sis) of, 1927. *M p. 840.* (Arch. Hydrobiol., 927, 81.)

FFERENTIATING CHARACTERS: Color- uniseriately multicellular organisms a spiral axis. Motile without flagella. n growing in the presence of H_2S sul- s deposited internally.

Type species: *Thiospirillopsis floridana* Uphof.

NOTES: A single species has been described. The organism is 2 to 3 μ wide and individual cells, 3 to 5 μ long.

Found in sulfur spring waters of Florida.

Thioploca (Thi.o.plo′ca) Lauterborn, 1907. *M p. 841.* (Ber. deut. botan. Ges., *25,* 1907, 238.)

DIFFERENTIATING CHARACTERS: Colorless, uniseriately multicellular, flexible organisms occurring in bundles in a common gelatinous sheath; motile without flagella; when growing in the presence of hydrogen sulfide they deposit sulfur inside the cells.

Type species: *Thioploca schmidlei* Lauterborn.

NOTES: Four species are listed. It is possible that they may be algae. Closely related blue-green algae such as *Hydrocoleus* and *Microcoleus* may appear very pale and deposit sulfur internally and thus resemble *Thioploca.*

Species differentiation is based upon size. Width varies from 1 to 9 μ.

Found in fresh waters and marine muds containing H_2S.

Thiothrix (Thi′o.thrix) Winogradsky, 1888. *M p. 842.* (Beitr. Morph. Physiol. Bakteriol., I, Schwefelbacterien, Leipzig, 1888, 39.)

DIFFERENTIATING CHARACTERS: Cells occur in nonmotile, segmented trichomes which are differentiated into base and tip and surrounded by a delicate sheath. Trichomes grow attached at the base to solid objects by means of gelatinous holdfasts. Reproduction is by transverse fission of the segments and by rod-shaped, so-called conidia which probably are the apical segments which become free. Temporarily the conidia show creeping motility, become attached to solid objects, and develop into new trichomes. When growing in the presence of H_2S the organisms deposit sulfur internally.

Type species: *Thiothrix nivea* (Rabenhorst) Winogradsky.

NOTES: There are seven listed species,

differentiated on the size of the trichome which varies in diameter from less than 1 μ to 30 μ. Some are halophilic.

Found in fresh and salt water containing H_2S.

Compare this genus with *Sphaerotilus.*

Vitreoscilla (Vit.re.os.cil′ la) Pringsheim, 1949. *M p. 845.* (Bacteriol. Revs., *13*, 1949, 70; see also J. Gen. Microbiol., *5*, 1951, 127 and 147.)

DIFFERENTIATING CHARACTERS: Colorless, Gram-negative, uniseriately multicellular* organisms, 2.0 μ wide or less, which are motile by a gliding action; not flagellated; *aerobic;* do not deposit sulfur internally when exposed to hydrogen sulfide.

Type species: *Vitreoscilla beggiatoides* Pringsheim.

NOTES: There are nine species listed of which one is claimed to be nonmotile. There are two principal morphological forms: (*a*) those in which the cells are cylindrical and square-ended and closely abutted to form a continuous thread with parallel sides and (*b*) those in which the individual cells are barrel-shaped, giving the thread a beaded appearance. The latter type shows a marked tendency to break up into hormogonia-like fragments whereas the former produces continuous threads. End cells are usually rounded but may be pointed (*V. conica*). None of the described species exceeds 2.0 μ in width. The tips of the threads may oscillate during movement.

Growth occurs readily on several types of organic media provided the con tion of ingredients is not too high. centration of 0.1 to 0.3 per cent pep adequate. The pH should not be be and the agar not too hard. Colon colorless and characteristic of glidi ganisms. They are usually flat an like as a result of the migration and of the gliding organisms. At the ma colonies short or long threads are invariably found migrating out ah the main growth, a characteristic ra ever seen with even actively motile lated bacteria.

Differentiation of species is at based solely upon the width of tri and size and shape of the individua

Found in dung and rotting ve matter, particularly in damp situat

Bactoscilla (Bac.tos.cil′ la) heim, 1949. *M p. 848.* (Bacteriol. *13*, 1949, 72; J. Gen. Microbiol., *5*, 144.)

DIFFERENTIATING CHARACTERS: less, rod-shaped organisms, less tha wide, arranged in long chains whi motile by a gliding motion on sol faces; not flagellated; Gram-negativ

Type species: *Bactoscilla flexibilis* heim, 1949.

NOTES: A single species is des Individual cells are 0.4 to 0.5 μ wid are comparatively rigid. Bending chains occurs at the junction betwe rods.

Microscilla (Mic.ros.cil′ la) heim, 1951. *M p. 849.* (Bacteriol. *13*, 1949, 72; J. Gen. Microbiol., *5*, 127 and 140.)

DIFFERENTIATING CHARACTERS: Fr ing, colorless, unicellular, filamentou ible microorganisms less than 2 μ wid more than 10 μ long; arranged motile by gliding action on a solid su not flagellated; Gram-negative; *a* heterotrophic. Do not form micro Not known to deposit iron.

Type species: *Microscilla marina* heim.

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* Multicellularity similar to that found in some blue-green algae (*Oscillatoria, Anabaena,* and others) is assumed to exist in the genus *Vitreoscilla* as evidenced by the key given in the *Manual* to differentiate the genus *Vitreoscilla* from *Bactoscilla. Vitreoscilla* is described as "trichomes divided into cells which are not separated by empty interspaces" as opposed to *Bactoscilla* which is described as "trichomes divided into cells separated by empty interspaces." This difference needs to be carefully checked. In the Comprehensive Key *Bactoscilla* has been inserted in both the *multicellular* and *unicellular* sections.

ES: Differentiation of these cells hose of *Cytophaga* apparently rests n size and their extremely rapid nent. Cells range from 10 to 100 μ gth, depending on the species.

ee species are described, one of ma- nd two of fresh water origin. Growth on 0.1 per cent peptone or yeast t agar with sea water for the marine s. Colonies are colorless or orange, icroscopic examination of the margins s advancing tongues of actively glid- ganisms.

icothrix (Leu′ co.thrix) Oersted, emend. Harold and Stanier, 1955. *850.* (Oersted, De regionibus marinis, nta topographiae historiconaturalis Oeresund, Copenhagen, 1844, 44; d and Stanier, Bacteriol. Revs., *19*, 54; *Pontothrix* Nadson and Krassil- , Compt. rend. acad. sci. U. R. S. S., o. 1, 1932, 243.)

FERENTIATING CHARACTERS: Color- uniseriately multicellular organisms n taper from the base to the apex and ttached at the basal end to a solid ob- Single, nonflagellated cells separate the apex and are reputed to glide, rosettes, produce holdfasts, and de- into new threads. *Aerobic.* Sulfur is deposited internally with growth in resence of hydrogen sulfide.

pe species: *Leucothrix mucor* Oersted.

TES: Harold and Stanier record no sheath. They claim, however, that their organism is identical with *Pontothrix* which was originally recorded as encased in a sheath several microns wide. Pringsheim (Bacteriol. Revs., *21*, 1957, 69) states that *Leucothrix* has a sheath.

The single species studied is halophilic and develops best in 16 per cent salt on rotting marine algae. Optimal temperature, 25° C.

Achromatium (A.chro.ma′ ti.um) Schewiakoff, 1893. *M p. 852.* (Über einen neuen bacterienähnlichen Organismus des Süsswassers, Habilitationsschrift, Heidelberg, 1893.)

DIFFERENTIATING CHARACTERS: Large, unicellular organisms, more than 2 μ wide, which are spherical to short cylindrical with hemispherical ends. They exhibit a slow, jerky, rolling motion. No flagella have been demonstrated. They divide by constriction. In their natural habitat they contain sulfur and large spherules of calcium carbonate, but both are environmental characters.

Type species: *Achromatium oxaliferum* Schewiakoff.

NOTES: Two species have been described, one from fresh and brackish mud and one from marine mud. Cells vary in size from spheres 5 to 7 μ in diameter to large forms 35 to 100 μ. They have never been isolated.

THE SLIME BACTERIA

NERAL CHARACTERS: The vegetative of slime bacteria are flexible, Gram- tive rods of low refractive index which without exception, motile by a slow ng movement on solid surfaces. Be- e of this the colonies produced are ini- y flat, and at the margins single cells oups of cells can be observed migrating y from the main body of cells—fre- ntly leaving a visible trail of slime be- l. Cells which have advanced some considerable distance may return along the same trail to join the main mass of cells. See Plate 27, Fig. 1a.

Vegetative cells are of two types. One is rod-shaped with blunt rounded ends and resembles that of ordinary bacteria. The other is fusiform and usually more flexible than the rod form.

No cell wall similar to that found with the flagellated bacteria is demonstrable by staining methods. There are no flagella. At present any suggestions as to the mode of locomotion are purely speculative.

Resting cells are produced by all genera other than *Cytophaga*. They occur in fruiting bodies of various types in all other genera except *Sporocytophaga* in which they occur among the rod forms within the colonies.

Each resting cell is formed from a single cell. In some the cells merely become shorter and thicker. In others the rods transform into spherical or elliptical resting cells. These are surrounded by a refractile, deeply staining wall and are referred to as microcysts.

The type of fruiting body varies with different genera. Resting cells may occur within cysts in the fruiting body or remain separated but embedded, as a rule, in a mass of slime. Fruiting bodies may be sessile or stalked.

The slime bacteria are commonly found in the decomposing dung of herbivorous animals and decaying plant materials in general. Some are of marine origin and digest chitin and agar. Many terrestrial forms are active cellulose digesters. Some genera, like *Cytophaga*, *Sporocytophaga*, and *Myxococcus*, are readily cultured on cellulose or bacterial cell agars. Many others, although producing fruiting bodies on sterile dung pellets layered on moist soil, have proved refractory to further culture.

Cytophaga (Cy.to′pha.ga) Winogradsky, 1929. *M p. 858.* (Ann. inst. Pasteur, *43*, 1929, 578.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, flexible, rod-shaped organisms less than 2 μ wide and 10 μ or less in length; arranged singly; motile by a gliding motion on solid surfaces; not flagellated; Gram-negative; *aerobic*; heterotrophic; no microcysts or resting cells are formed. Not known to deposit iron.

Type species: *Cytophaga hutchinsonii* Winogradsky.

NOTES: Eleven species are listed, three of which are obligate halophiles. They are fusiform or round-ended rods varyin 0.3 to 1.0 μ wide and, on the averag 10 μ long. Two species extend to 20 *Microscilla*). In old cultures dege coccoid forms, not microcysts, are f

Nine of 11 species have been cultu various media including filter pape gels, glucose-mineral salts media, agar, peptone agar, and sea water-p agar.

In the more restrictive media gro in the form of a barely visible fil etching on the surface. On richer raised moist colonies may be pro Colonies are usually pigmented y orange, pink, olive green, or gray.

Agar is hydrolyzed by marine s particularly.

Four species examined utilize but produce acid from a wide range of hydrates. Heated carbohydrates are toxic.

Of six species tested four use NH NO_3 for nitrogen. All six use pepton yeast extract.

Cellulose is hydrolyzed by 7/8 sp Ability to hydrolyze precipitated cel in agar plates is dependent upon agar ciently soft to permit migration of th through the medium.

Gelatin is liquefied by 4/5 species

Information on other biochemical is too fragmentary to record.

Aerobic; optimal temperature, 2 30° C.

Found in rotting vegetable matt soil and water, both fresh and marine

Archangium (Ar.chan′gi.um) 1924. *M p. 863.* (Beitr. botan. Protis gie. I, Die Polyangiden. Geb. Borntra Leipzig, 1924, 67.)

DIFFERENTIATING CHARACTERS: Fre ing, unicellular, nonphotosynthetic, ible, rod-shaped microorganisms, 0.5 t μ by 4 to 10 μ; motile by a gliding m on solid surfaces. Fruiting bodies formed in which cylindrical resting are produced in a mass of convo tubules embedded in slime; not in de *aerobic*; heterotrophic; Gram-nega- ot known to deposit iron.

e species: *Archangium gephyra* Jahn.

ES: Five species are listed, none of appears to have been isolated in pure e. Descriptions are based mainly on pe of fruiting bodies observed on dung or rotting vegetable matter. The s, the mass of which forms the fruit- dy, vary in width from 40 to 90 μ ay be incompletely septate. The con- d mass of tubules gives the fruiting an irregular surface. The fruiting are sessile and yellow, orange or red r and approximately 0.5 to 1 mm. in ; distinguished from *Stelangium* by pressed nature of the fruiting bodies. rod-shaped resting cells, which are er and wider than the corresponding ative cells, are packed within the es.

und in soil. Fruiting bodies have been l mainly on rotting animal excreta.

langium (Ste.lan′gi.um) Jahn, *M p. 866*. (Kryptogamenflora der Brandenburg, V, Pilze I, Lief 2, 1915,

FFERENTIATING CHARACTERS:* Free- g, unicellular, nonphotosynthetic, flex- rod-shaped microorganisms less than ide; arranged singly; motile by a glid- notion on solid surfaces; no flagella; drical resting cells are produced within right finger-like, sessile fruiting body ncysted; *aerobic*; heterotrophic; Gram- ive; not known to deposit iron.

pe species: *Stelangium muscorum* xter) Jahn.

TES: There is only one species listed. fruiting body is a bright orange-yellow, o 300 μ long and 10 to 50 μ wide, and sionally forked. Resting cells are 1 to μ by 4 to 6 μ. Found on liverworts.

Sorangium (So.ran′gi.um) Jahn, 1924. *M p. 866*. (Beitr. botan. Protistologie. I, Die Polyangiden. Geb. Borntraeger, Leipzig, 1924, 73.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, flexible, rod-shaped microorganisms *with blunt rounded ends*; less than 2 μ wide; arranged singly; motile by a gliding motion on solid surfaces. Fruiting bodies are produced in which rod-shaped resting cells are formed in well defined cysts that are *predominantly angulated* but may be spherical or cylindrical. Cysts are grouped in slime envelopes to form the fruiting bodies which are sessile; *aerobic*; heterotrophic; Gram-negative; not known to deposit iron.

Type species: *Sorangium schroeteri* Jahn.

NOTES: Eight species are listed. The vegetative cells vary from 0.4 to 1.3 μ wide and 2.0 to 6.0 μ long, according to species.

Fruiting bodies may be limited to small groups of cysts distributed throughout decomposing cellulose fibers (*S. nigrum*) or organized structures containing large numbers of cysts and measuring from 0.05 to 1 mm. wide. Cysts are described as angular for only four of the eight species. The others are cylindrical to spherical. They vary in size with species from forms 1.6 to 3.2 μ wide containing only 10 resting cells (*S. cellulosum*) to forms 18 to 22 μ by 12 to 22 μ. Colors are bright orange, brown, yellow, or black. They are embedded in slime. Cysts are sometimes grouped as primary and secondary cysts within a sorus.

The resting cells are shorter and thicker than the vegetative forms. They average 0.3 to 0.8 μ by 3 to 5 μ for listed species.

Found in rotting dung, soil, and decaying vegetation.

Polyangium (Po.ly.an′gi.um) Link, 1809. *M p. 870*. (Mag. Ges. Naturforsch. Feunde Berlin, *3*, 1809, 42.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, flexible, rod-shaped microorganisms less than 2 μ wide; arranged singly; motile by a gliding motion on solid surfaces; no flagella.

Resting cells but not vegetative cells been described. The statement regard- he vegetative cell is hypothetical but sed on the measurements given for the ng cells and the general characteristics yxobacteria.

Resting cells produced in cysts which are spherical or elongate and coiled. Cysts are embedded in slime to form a sorus which is sessile. *Aerobic;* heterotrophic; Gram-negative; not known to deposit iron.

Type species: *Polyangium vitellinum* Link.

NOTES: Although the *Manual* key to families suggests a predominance of long tapering rods, seven species for which this character is mentioned are described as cylindrical rods with *rounded ends.* They vary in size from 0.4 to 1.2 μ wide (average 0.8 μ) by 3 to 12 μ long.

Cysts are predominantly spherical or cylindrical. *P. cellulosum* is described as having polygonal cysts caused by pressure on cylindrical cysts. Cysts vary in size from 8 to 200 μ in width, according to species. They may be yellow, orange, rose, brown, or smoky gray in color.

Fruiting bodies formed from cyst aggregates vary from approximately 0.1 to 3 mm. in width and are sessile. Resting cells are rod-shaped and shorter than the vegetative cells.

Fifteen species have been recorded.

Found in damp, rotting timber, soil, and dung.

Synangium (Syn.an′ gi.um) Jahn, 1924. *M p. 877.* (Beitr. botan. Protistologie. I, Die Polyangiden. Geb. Borntraeger, Leipzig, 1924, 79.)

DIFFERENTIATING CHARACTERS: There is a single species listed. The vegetative rods are 0.9 to 1.0 μ by 3 to 8 μ with rounded ends. They are motile by a gliding action on solid surfaces; no flagella. Resting cells, 2.5 to 6.0 μ by 0.6 to 1.0 μ, are produced in cysts which have apical hair-like processes 7 to 30 μ long and which are attached at the base to form a discoid or spherical cluster supported on a stalk up to 1 mm. high. *Aerobic;* heterotrophic; Gram-negative; not known to deposit iron.

Type species: *Synangium sessile* (Thaxter) Jahn.

NOTES: Initially white, the fruiting body finally turns an orange color.

Found in decaying wood. Crude cul
have been maintained on hay.

Podangium (Po.dan′ gi.um)
1924. *M p. 877.* (Beitr. botan. Protis
gie. I, Die Polyangiden. Geb. Borntra
Leipzig, 1924, 80.)

DIFFERENTIATING CHARACTERS: Fre
ing, unicellular, nonphotosynthetic,
ible, rod-shaped microorganisms, less
2 μ wide, arranged singly; 2 to 7 μ
motile by a gliding action on solid surf
no flagella. Resting cells are rod-sh
and are produced in spherical, cylin
or disc-shaped cysts which are born s
on stalks; *aerobic*; heterotrophic; G
negative; not known to deposit iron.

Type species: *Podangium ere*
(Schroeter) Jahn.

NOTES: Four species are described.
are, on the average, 25 to 50 μ wide.
disc-shaped cyst of *P. boletus* is 100
to 50 μ. Stalks range from 7 to 80 μ
may be square or round and somet
have a distinct foot. Resting cells are
shaped.

Fruiting bodies are white, yellow, or
or brown.

Chondromyces (Chon.dro′ my.ce
Chon.dro.my′ ces) Berkeley and C
1874. *M p. 879.* (Berkeley, Notes on N
American Fungi, Grevillea, *3*, 1874, 9

DIFFERENTIATING CHARACTERS: Fre
ing, unicellular, nonphotosynthetic,
ible, rod-shaped microorganisms, less
2 μ wide; arranged singly; motile by a
ing motion on solid surfaces. Rod-sh
resting cells are produced in cysts, se
of which are borne on a common s
Aerobic; heterotrophic; Gram-negative
known to deposit iron.

Type species: *Chondromyces croc*
Berkeley and Curtis.

NOTES: Eight species are described.
vegetative rods measure from 0.5 to
wide by 2 to 11 μ long. Ends are rou
or pointed.

The cysts vary considerably in shap
cording to species. They may be spi
shaped, bell-shaped, conical, cylindrica

cal. They vary from 9 to 80 μ wide 6 to 100 μ long. The majority are a orange in color. The cysts are at-l by pedicles to the main stalk. The may be more than 0.5 mm. high. *hondromyces catenulatus* cysts are in chains and several chains are at-l by one end to the common stalk.

nd on rotting vegetable matter.

xococcus (Myx.o.coc′ cus) Thaxter, *M p. 883.* (Botan. Gaz., *17*, 1892,

FERENTIATING CHARACTERS: Free-liv- unicellular, nonphotosynthetic, flex- rod-shaped microorganisms less than de; arranged singly; motile by a gliding motion on solid surfaces; no flagella. rical or elliptical resting cells (micro- are produced by transformation of ods but are *not contained in cysts.* ing bodies are formed by the segrega- of the rod-shaped cells with the for- on of a raised spherical, conical, or shaped structure, sessile or on a broad stalk, in which the microcysts are dded in slime. *Fruiting bodies* are uescent. *Aerobic*; heterotrophic; n-negative; not known to deposit iron.

pe species: *Myxococcus fulvus* (Cohn, d. Schroeter) Jahn.

TES: Nine species are listed of which ave been recognized. The vegetative are pointed or rounded and measure ximately 0.4 to 1.3 μ by 2 to 10 μ. *microcysts* are spherical or elliptical, o 1.2 μ by 1.0 to 1.8 μ. Some have y refractile walls. All stain intensely. fruiting bodies may be more than 0.5 in size and are white, orange, red, or w-green in color.

od growth of some species has been ved on bacterial cell agar, cellulose , and nutrient agar.

und in decaying vegetable matter.

ondrococcus (Chon.dro.coc′ cus) , 1924. *M p. 886.* (Beitr. botan. Pro- ogie. I, Die Polyangiden. Geb. Born- er, Leipzig, 1924, 85.)

FERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, flexible, rod-shaped microorganisms, less than 2 μ wide; arranged singly; motile by a gliding motion on solid surfaces; no flagella; spherical or elliptical resting cells (microcysts) are produced by transformation of the rods but they are *not contained in cysts.* Fruiting bodies, formed by the segregation and transformation of the rods to microcysts, are *firm and not deliquescent. Aerobic*; heterotrophic; Gram-negative; not known to deposit iron.

Type species: *Chondrococcus coralloides* (Thaxter) Jahn.

NOTES: Seven species are described. The vegetative rods are 0.4 to 0.8 μ wide and 2 to 12 μ long. Microcysts are 1 to 2 μ in diameter or approximately 0.7 by 1.2 μ. Some have thick refractile walls. The fruiting body varies with the species. In some it is a depressed disc-like structure, lobed or convoluted, and 80 by 160 μ in size. In others it may have a swollen base and a narrow tip like a partly inflated balloon. Occur singly or in groups. Colors are pink, red, yellow, or violet. Some fruiting bodies are more than 0.5 mm. across.

Found on dung of animals and birds and on lichens.

Angiococcus (An.gi.o.coc′ cus) Jahn, 1924. *M p. 889.* (Beitr. botan. Protistologie. I, Die Polyangiden. Geb Borntraeger, Leipzig, 1924, 89.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, flexible, rod-shaped microorganisms, less than 2 μ wide; arranged singly; motile by a gliding motion on solid surfaces; no flagella. Spherical *microcysts* are *produced in disc-shaped cysts* which are aggregated to form a fruiting body; *aerobic*; heterotrophic; Gram-negative; not known to deposit iron.

Type species: *Angiococcus disciformis* (Thaxter) Jahn.

NOTES: There are two species. The vegetative cells are 0.4 to 0.6 μ by 1.5 to 3.0 μ. The disc-like cysts are 10 to 20 μ thick and 35 to 150 μ in diameter, and are yellow, orange, or pink in color.

Found on decaying organic matter.

Sporocytophaga (Spo.ro.cy.to′pha.ga) Stanier, 1940. *M p. 890.* (J. Bacteriol., *40*, 1940, 629.)

DIFFERENTIATING CHARACTERS: Free-living, unicellular, nonphotosynthetic, Gram-negative, flexible, rod-shaped microorganisms, motile by a gliding motion on solid surfaces; no flagella; microcysts are produced by direct transformation of rods to spherical or elliptical resting cells *without aggregation into* a fruiting body; *aerobic*; heterotrophic; Gram-negative; not known to deposit iron.

Type species: *Sporocytophaga myxococcoides* (Krzemieniewska) Stanier.

NOTES: Three species are listed. The are 0.3 to 0.7 μ by 2.5 to 8.0 μ and flexible. No cell wall is revealed by s stains.

The colonies on agar vary with th tritional quality of the medium from etch-like growth to a raised, moist, sp ing growth. The colonies are yello orange.

Microcysts are spherical or oval an cur among the rods in the colonies.

All species use ammonia, nitrate, peptone for a nitrogen source. Cellul hydrolyzed. Glucose is metabolized i heated.

The optimal temperature is 25° to 3 Found in soil and composts.

EXTREMELY FLEXIBLE SPIRAL ORGANISMS ACTIVELY MOT IN FREE SOLUTION BUT WITHOUT FLAGELLA; AXIAL FILAME REVEALED BY ELECTRON PHOTOMICROGRAPHS IN ALL TY SO FAR EXAMINED

Spirochaeta (Spi.ro.chae′ ta) Ehrenberg, 1833. *M p. 893.* (Abhandl. Berlin Akad., 1833, 313.)

DIFFERENTIATING CHARACTERS: Free-living, colorless, unicellular, flexible microorganisms with a spiral axis, 0.25 to 0.75 μ wide and *exceeding 30 μ in length.* Wound around the main spiral of the cell is a small group of fine fibrils having the appearance of a single *axial filament* under the ordinary microscope. Very actively motile and exhibiting remarkable flexibility; no flagella; no cross striations; do not deposit sulfur internally; Gram-negative.

Type species: *Spirochaeta plicatilis* Ehrenberg.

NOTES: Five species are described, differentiated solely on the basis of size and the shape of the ends which may be rounded or pointed. Some species exceed 300 μ in length. The cells stain violet with Giemsa. The apparent axial filament observed with the standard microscope is composed of a number of filaments.

Found in waters, particularly amon composing plant residues.

Saprospira (Sap.ro.spi′ ra) Gross, *M p. 894.* (Mitt. Zool. Stat. Neape 1911, 190).

DIFFERENTIATING CHARACTERS: Fre ing, colorless, flexible microorganisms a spiral axis, 0.5 to 1.2 μ wide and ex ing 30 μ in length. There are no visibl ments wound around the main axis. cells *appear to be* partitioned by cross Motile with a rotating action; no fla do not deposit sulfur internally.

Type species: *Saprospira grandis*

NOTES: Three species are reco Means for differentiation are not give would appear to be based upon size, amplitude, and width.

Found in foraminiferous sand an oysters.

Not yet examined by the electron m scope (1958).

Cristispira (Cris.ti.spi′ ra) Gross,

895. (Mitt. Zool. Stat. Neapel, *20*,
41.)

ERENTIATING CHARACTERS: Free-liv-
olorless, Gram-negative, flexible mi-
anisms with a spiral axis; 0.5 to 3.0 μ
and *exceeding 30 μ in length*. Wound
d the main spiral is a group of ap-
nately 40 fibrils forming a colorless
alled a crista, which stands out from
ll. Fibrils are not visible under the
nicroscope but are visible under phase
ast (author's note). The crista, com-
of these fibrils, is visible with the
microscope. Cross striations are pres-
o not deposit sulfur internally.

pe species: *Cristispira balbianii* (Cer-
ollins.

ES: Three species are described. Dif-
iation is on size, shape of the ends,
he spiral width and amplitude.

und in the crystalline style of oysters
ther bivalves.

rrelia (Bor.rel′i.a) Swellengrebel,
M p. 897. (Ann. inst. Pasteur, *21*,
582.)

FERENTIATING CHARACTERS: Free-liv-
colorless, unicellular, flexible microor-
ms with a shallow spiral axis; 0.25 to
wide and 8 to 16 μ long; motile with-
agella; no fibrils are visible around the
with a light microscope, but they are
led by electron microscopy. *Stain*
y; Gram-negative.

pe species: *Borrelia anserina* (Sak-
) Bergey *et al.*

TES: Of 28 species recorded, only eight
to have been cultured. Differentiation
sed solely on source, mode of trans-
on, and the nature of the infection.
species are hematophytic or occur on
us membranes.

any species cause relapsing fever in

ptospira (Lep.to.spi′ra) Noguchi,
M p. 907. (J. Exptl. Med., *25*, 1917,

FERENTIATING CHARACTERS: Free-liv-
nicellular, colorless, flexible organisms,
0.2 μ thick, wound in a very fine coil
and hooked at one or both ends. A single axial filament, visible only with the electron microscope, is wound around the main coils of the organism; *aerobic*; can be cultivated *in vitro* in semisolid rabbit plasma media; not readily stained; stain with Giemsa's stain or by silver impregnation methods; visible under dark ground illumination. Actively motile in free solution.

Type species: *Leptospira icterohaemorrhagiae* (Inada and Ido) Noguchi.

NOTES: The *Manual* lists only two species, one, *L. icterohaemorrhagiae*, representative of the parasitic species, and *L. biflexa*, representative of the saprophytic species.

Numerous serotypes of the pathogenic leptospirae have been identified by agglutination-lysis and cross absorption tests.

Apart from size, there is no clear means of differentiation between *Leptospira* and *Spirochaeta*. Is it possible that a better mode of differentiation could be found in the number of axial filaments revealed by electron microscopy?

Treponema (Tre.po.ne′ma) Schaudinn, 1905. *M p. 904*. (Deut. med. Wochschr., *31*, 1905, 1728.)

DIFFERENTIATING CHARACTERS: Free-living, colorless, unicellular, flexible microorganisms with a spiral axis in which spirals are acute and regular or irregular; 0.25 to 0.3 μ wide and 3 to 18 μ long. Visible under dark ground illumination; no fibrils are visible under these conditions, but they have been demonstrated by electron microscopy (four in *T. pallidum*); do not stain by Gram; motile without flagella; *anaerobic*.

Parasities of man and animals.

Type species: *Treponema pallidum* (Schaudinn and Hoffmann) Schaudinn.

NOTES: Eight species are recorded, some of which have been cultured in sterile tissue cultures under strictly anaerobic conditions. Differentiation is based on morphology, source, and the nature of the infection, if any.

Certain species cause syphilis (*T. pallidum*), pinta, (*T. cuniculi*), and yaws (*T. pertenue*).

PLEUROPNEUMONIA AND PLEUROPNEUMONIA-LIKE ORGANIS

Mycoplasma (My.co.plas′ma) Nowak, 1929. *M p. 914.* (Ann. inst. Pasteur, *43*, 1929, 1349.)

DIFFERENTIATING CHARACTERS: Small, spherical bodies, 150 to 300 mμ in diameter, which germinate to produce filaments, approximately 0.2 μ wide and from 2 to 50 μ long, which are sparsely or richly branching. At a later stage of growth small endomycelial corpuscles develop in the filaments by a process of successive condensation and constriction. As a result the homogeneous filaments are retransformed into chains of close-set spherical bodies which are released by fragmentation; highly resistant to penicillin and sulfathiazole; colonies on agar have a dense granulated central area which penetrates into the agar and which is surrounded by a translucent flat peripheral zone or the colonies consist pearly film containing numerous spots to calcium or magnesium soaps; do ferment lactose, sucrose, mannitol dulcitol.

Type species: *Mycoplasma myc* (Borrel *et al.*) Freundt.

NOTES: Fifteen species are describ the *Manual*, separated on pathogeni hosts, and cultural and biochemical pr ties. L-phase colonies of some bacteria a strong resemblance to the coloni *Mycoplasma*. The latter are generally opaque, more heavily marked on the face, tend to revert to the normal baci form in penicillin-free semisolid media more difficult to subculture, do not re cholesterol for growth, and ferment same carbohydrates as the parent orga

OBLIGATE PARASITES OF THE ANIMAL KINGDOM

NOTE: The following genera have been placed in the order *Rickettsiales* in the *Manual*. See also Introduction.

Rickettsia (Ri.ckett′si.a) da Rocha-Lima, 1916. *M p. 935.* (Berlin. klin. Wochschr., *53*, 1916, 567.)

DIFFERENTIATING CHARACTERS: Small, often pleomorphic, rod-shaped to coccoid organisms which usually occur intracytoplasmically in lice, fleas, ticks, and mites. Occasionally occur extracellularly in gut lumen. Nonfilterable. Gram-negative. Have not been cultivated in cell-free media. Pathogenic species parasitic on man and other animals. Cause mild to severe typhus-like infections in appropriate vertebrate hosts of arthropod vectors; intracytoplasmic, occasionally intranuclear, in tissues. Etiological agents of epidemic typhus, murine or endemic typhus, Rocky Mountain spotted fever, tsutsugamushi disease, rickettsialpox, and other diseases. Many related organisms, described as symbiotes in arthropods not pathogenic for vertebrates, have been assigned here, but i mation is much less complete than fo pathogenic forms, and their congene with the type species is uncertain. Pl genetic relationships remain to be e lished.

Type species: *Rickettsia prowazeki* Rocha-Lima.

Coxiella (Co.xi.el′la) Philip, (1 1948. *M p. 947* (Subgenus *Coxiella* P Am. J. Hyg., *37*, 1943, 306; *Coxiella* P Public Health Repts. (U. S.), *63*, 1948,

DIFFERENTIATING CHARACTERS: S pleomorphic, rod-shaped or coccoid ganisms occurring intracellularly in cytoplasm of infected cells and pos extracellularly in infected ticks. Filter Stain lightly with aniline dyes. Gram-n tive. Have not been cultivated in cel media. Parasites of man and other anin Includes the etiological agent of Q f Produces no typhus-like rash or Felix titer in man. Not dependent o d transmission in the infectious

e species: *Coxiella burnetii* (Derrick)

lichia (Ehr.li′ chi.a) Moshkovskiy,
1945. *M p. 949.* (Subgenus *Ehrlichia*
kovskiy, Compt. rend. soc. biol., *126*,
382; Ehrlichia Moshkovskiy, Uspekhi
nennoĭ Biol., *19*, 1945, 10.)

ERENTIATING CHARACTERS: Small,
pleomorphic, usually coccoid organ-
occurring intracytoplasmically in the
ating monocytes of suitable mamma-
osts. Parasitic. The etiological agents
k-borne diseases of dogs, cattle, and
.

pe species: *Ehrlichia canis* (Donatien
Lestoquard) Moshkovskiy.

wdria (Cow′ dri.a) Moshkovskiy,
) 1947. *M p. 950.* (Subgenus *Cowdria*
kovskiy, Uspekhi Sovremennoĭ Biol.,
945, 18; *Cowdria* Moshkovskiy, Sci-
106, 1947, 62.)

FERENTIATING CHARACTERS: Small,
morphic, spherical or ellipsoidal, oc-
nally rod-shaped organisms occurring
cellularly in ticks and characteristi-
localized in clusters inside vacuoles in
ytoplasm of vascular endothelial cells
st vertebrates. Gram-negative. Have
een cultivated in cell-free media. Not
ovarially transmitted in tick vectors.
etiological agent of heartwater of
, sheep, and goats.

pe species: *Cowdria ruminantium*
dry) Moshkovskiy.

orickettsia (Ne.o.ri.ckett′ si.a)
et al., 1953. *M p. 951.* (Philip, Had-
and Hughes, Riassunti delle Comuni-
ni, 6th Congr. intern. microbiol.,
e, *2*, 1953, 256.)

FFERENTIATING CHARACTERS: Small,
oid, sometimes pleomorphic (in the
of short rods, crescents, and even
), intracytoplasmic organisms which
r in the reticuloendothelial cells of
in mammals and in tissues of at least
re fluke vectors. No intranuclear forms
been observed. Nonfilterable. Nonmotile. Not cultivable on cell-free media. The etiological agent of a helminth-borne disease of canines.

Type species: *Neorickettsia helminthoeca* Philip *et al.*

Wolbachia (Wol.ba′ chi.a) Hertig, 1936. *M p. 953.* (Parasitology, *28*, 1936, 472.)

DIFFERENTIATING CHARACTERS: Microorganisms possessing the general characteristics of the rickettsiae and exhibiting not only minute, bacterium-like forms appearing with dark field illumination as luminous rods and points but also enlarged forms within the body of which are contained one to several smaller individuals. Pleomorphism is characteristic, and it is usually found in organisms in an intracellular location; the few extracellular species, *e.g.*, *Wolbachia melophagi*, may eventually be regarded as not belonging to this genus.

Type species: *Wolbachia pipientis* Hertig.

Symbiotes (Sym.bi.o′ tes) Philip, 1956. *M p. 956.* (Can. J. Microbiol., *2*, 1956, 267.)

DIFFERENTIATING CHARACTERS: Rickettsia-like, pleomorphic organisms living chiefly intracellularly in arthropod tissues and approaching most nearly the true symbiotic or commensalistic relationship to their hosts; this is evidenced by the development in the host of special organs, or mycetomes, although no species of the *Rickettsiales* is yet known to be confined to such a location.

Type species: *Symbiotes lectularius* (Arkwright *et al.*) Philip.

Rickettsiella (Ri.ckett.si.el′ la) Philip, 1956. *M p. 957.* (Can. J. Microbiol., *2*, 1956, 267.)

DIFFERENTIATING CHARACTERS: Minute, intracellular, rickettsia-like organisms which are pathogenic for certain insect larvae but which are not known to be pathogenic for any vertebrates. Filterable. Associated with microscopic, intracellular, crystalline inclusions and reported to infect cell nuclei (although this needs to be confirmed).

Type species: *Rickettsiella popilliae* (Dutky and Gooden) Philip.

Chlamydia (Chla.my′ di.a) Rake, 1956. *M p. 958.* (*Prowazekia* Coles, Ann. N. Y. Acad. Sci., *56*, 1953, 461; *Chlamydia* Rake, Bergey's Manual of Determinative Bacteriology, 7th ed., 1957.)

DIFFERENTIATING CHARACTERS: Coccoid and spherical cells with a developmental cycle. Gram-negative. Occur intracytoplasmically. Noncultivable in chicken embryonic tissues. Have not yet been cultivated in tissue culture. Susceptible to the action of sulfonamides and of antibiotics. Cause ophthalmic and urogenital diseases in man; transferable to other primates.

Type species: *Chlamydia trachomatis* (Busacca) Rake.

Colesiota (Co.le.si.o′ ta) Rake, 1948. *M p. 959.* (Bergey's Manual of Determinative Bacteriology, 6th ed., 1948, 1119.)

DIFFERENTIATING CHARACTERS: Usually coccoid cells, but pleomorphism is marked. Gram-negative. Occur intracytoplasmically as colonies. Cause ophthalmic diseases in sheep.

Type species: *Colesiota conjunctivae* (Coles) Rake.

Ricolesia (Ri.co.le′ si.a) Rake, gen. nov. *M p. 959.*

DIFFERENTIATING CHARACTERS: Usually coccoid cells, but pleomorphism is marked. Gram-negative. Occur intracytoplasmically as scattered growth. Cause ophthalmic diseases of warm-blooded animals.

Type species: *Ricolesia pullorum* (Zhdanov and Korenblit) Rake.

Colettsia (Co.lett′ si.a) Rake, nom. nov. *M p. 961.*

DIFFERENTIATING CHARACTERS: Large, pleomorphic cells occurring intracytoplasmically. Apparently nonpathogenic but may be saprophytic. Found only in the conjunctival cells of goats, sheep, and cattle.

Type species: *Colettsia pecoris* Rake.

Miyagawanella (Mi.ya.ga.wa.nel′ la) Brumpt, 1938. *M p. 961.* (Ann. parasitol. humaine et comparée, *16*, 1938, 153.)

DIFFERENTIATING CHARACTERS: Coccoid cells with a developmental cycle. Oc
tracytoplasmically. Gram-negative.
tivable in chicken embryonic tiss
tissue culture. Susceptible to sulfon
and antibiotics. Pathogenic, causing
ous diseases in warm-blooded animal

Type species: *Miyagawanella ly*
granulomatosis Brumpt.

Bartonella (Bar.to.nel′ la) S
Tyzzer, and Sellards, 1915. *M p. 9*
Am. Med. Assoc., *64*, 1915, 808.)

DIFFERENTIATING CHARACTERS: Mi
ganisms which multiply in fixed tiss
and on erythrocytes. On the red bloo
in stained films, the organisms app
rounded or ellipsoidal forms or as s
straight, curved, or bent rods occ
either singly or in groups. Characteris
occur in chains of several segmenti
ganisms, sometimes swollen at one o
ends and frequently beaded (Strong
J. Am. Med. Assoc., *61*, 1913, 1715),
out a distinct differentiation of nucle
cytoplasm. In the tissues they are si
within the cytoplasm of endothelia
as isolated elements and are grou
rounded masses. Possess independen
tility. Reproduce by binary fission.
be cultivated by unlimited serial tr
on cell-free media. Occur spontaneou
man and in arthropod vectors. One s
has been recognized, and it is known
established only on the South Am
continent and perhaps in Central Am
Human bartonellosis may be mani
clinically by one of the two syndrome
stituting Carrión's disease (Oroya fe
verruga peruana) or by an asympto
infection.

Types species: *Bartonella bacilli*
(Strong *et al.*) Strong *et al.*

Grahamella (Gra.ha.mel′ la) Br
1911. *M p. 971.* (Bull. soc. patho
otique, *4*, 1911, 514.)

DIFFERENTIATING CHARACTERS:
organisms occurring within the er
cytes of lower mammals. Morpholog
these organisms bear a resemblance
are less polymorphic than the spec

ella and stain more deeply with
's stain than do the bartonellae.
r motility nor flagella have been
strated. Not acid-fast. Gram-nega-
everal species have been cultivated
living media. Growth is favored by
ldition of hemoglobin. In cultures,
light propensity to grow in un-
ed filaments is variable; rods and
ds with indistinct contours are com-
cemented together in dense masses.
ic. Parasitic. Splenectomy has little
on the course of infection. Nonpatho-
Not affected by arsenicals. The eti-
al agent of grahamellosis of rodents
some other vertebrates.

pe species: *Grahamella talpae* Brumpt.

emobartonella (Hae.mo.bar.to.
) Tyzzer and Weinman, 1939. *M p.*
Am. J. Hyg., *30*, 1939, 141.)

FERENTIATING CHARACTERS: Parasites
red blood cells. There is no demon-
e multiplication in tissues, and cu-
us eruptions are not produced. Typi-
rod-shaped or coccoid organisms
show no differentiation into nucleus
ytoplasm. The morphological range
vary with the type of host employed.
well with Romanowsky-type stains
oorly with many other aniline dyes.
cid-alcohol-fast. Gram-negative. Not
ated indefinitely in cell-free media.
buted over the surface of the erythro-
and possibly sometimes within them.
y produce disease in animals without
ectomy. The experimental host range
ricted; an organism infective for one
s of rodent may commonly infect
rodents but not primates. Markedly
nced by arsenotherapy but, so far as
own, do not respond to penicillin.
r naturally as parasites of vertebrates.
smitted by arthropods. Geographical
bution of the best studied species is
ar to that of the vertebrate host and
be world-wide.

pe species: *Haemobartonella muris*
er) Tyzzer and Weinman.

erythrozoon (Ep.e.ryth.ro.zo′on) Schilling, 1928. *M p. 977.* (Klin. Wochschr., *72*, 1928, 1854.)

DIFFERENTIATING CHARACTERS: Microorganisms found in blood plasma and on erythrocytes. Stain well with Romanowsky-type dyes and then appear as rings, coccoids, or short rods, 1 to 2 μ in greatest dimension and stain bluish or pinkish violet; show no differentiation of nucleus and cytoplasm with this technique. Not acid-alcohol-fast. Gram-negative. Not cultivated in cell-free media. Splenectomy activates latent infection. Arthropod transmission has been established for one species.

The organisms in this genus have been considered as belonging to the *Protozoa* by Neitz, Alexander, and du Toit (Onderstepoort J. Vet. Research, *3*, 1934, 268) and to the bacteria by Mesnil (Bull. soc. pathol. exotique, *22*, 1929, 531) and by Tyzzer (in Weinman, Trans. Am. Phil. Soc., 1944, 244). The evidence at hand favors the inclusion of this group among those organisms which are not clearly protozoan in nature but which appear to be closely related to the bacteria.

Type species: *Eperythrozoon coccoides* Schilling.

Anaplasma (A.na.plas′ma) Theiler, 1910. *M p. 981.* (Rept. Gov. Vet. Bacteriol. for 1908–1909, Dept. Agr., Transvaal, 1910, 7.)

DIFFERENTIATING CHARACTERS: Organisms which parasitize red blood cells. There is no demonstrable multiplication in other tissues. In blood smears fixed with May-Grünwald and stained with Giemsa's stain, these organisms appear in the erythrocytes as spherical chromatic granules which stain a deep reddish violet. No differentiation into nucleus and cytoplasm is shown. Occur naturally as parasites of ruminants. Transmitted by arthropods. Situated at or near the margin and/or at or near the center of the red blood cells. The position within the erythrocyte or host differences or both factors serve as bases for differentiating species. Attempts at cultivation in a variety of media have failed. Produce disease in nonsplenectomized and in splenectomized ruminants. The natural and experimental host range is fairly wide; these organisms occur in members of the families *Bovidae* and *Camelidae.* Influenced by Aureon and Terramycin. Widely distrib throughout the world.

Type species: *Anaplasma marg* Theiler.

METHODS

Pope and Skerman Mineral Salts ia for the Cultivation of Auto- hs and Nonexacting Heterotrophs

ıe authors have failed to find any sible reason for the very large number fferent formulas given for the prepara- of inorganic media. In most, certain tituents have a specific function, but majority of the constituents are ob- sly intended to provide trace elements do so in quantities far in excess of the irements of the organisms. Phosphates usually included to provide a buffer and, minor extent, to provide phosphate for abolic purposes. The former function very doubtful significance in other than fixing of the initial pH of the medium; e in most cases the main criterion is vth, and a perceptible turbidity is ap- ent as a rule before any marked meta- c changes occur. Only when the meta- c byproducts are important may there any reason for strong buffering of the lium, and even in such cases the de- oility of it is questionable.

xcessive phosphate inevitably results he precipitation of large quantities of luble phosphates with the resultant ding of the medium which makes it ess when development of turbidity is main index of growth.

ı the formulation of the following basal lium, one of the most complex yet pub- ed, that of Meikeljohn for *Nitrosomonas* Gen. Microbiol., *4*, 1950, 185) which in- ved use of the Hoagland and Snyder -Z" trace element mixture, was critically mined with the view to retaining all elements represented but eliminating precipitate which forms. The investiga- ı was aided by the chance observation t yeasts grow well in Wickerham's de- d medium for yeasts (Northern Regional earch Laboratories Tech. Bull. No. 1029) with the phosphate reduced from 1 gm. to 1 mg. per L.

For the preparation of the various mineral salts media the solutions described in Tables I and II are required. They should all be prepared with glass-distilled water and acid-cleaned glassware.

PREPARATION OF THE BASAL MINERAL SALTS MEDIUM

Step 1: Pipette into a 1-L. standard flask the following amounts of solutions from Group B: 10.0 ml. of Solutions 1 and 2 and 0.1 ml. of each of Solutions 3 through 10.

Step 2: Add approximately 600 ml. of 0.0074 M H_3PO_4 (Solution 3, Group A) and 210 ml. of water.

Step 3: Adjust the pH to 7.0 with N NaOH (1, Group A).

Step 4: Add 0.1 ml. of Solutions 11 and 12 from Group B.

Step 5: Take 0.1 ml. of the $MnCl_2$ solution (13, Group B), add 9.9 ml. of 0.074 M H_3PO_4 (2, Group A), and adjust the pH to 7.0. Autoclave and filter. Add the filtrate to the medium.

Step 6: Add 10 ml. of Solution 14, Group B and 0.1 ml. of 15 and 16, Group B.

Step 7: Using the neutralized 0.0074 M H_3PO_4 (Solution 4, Group A), make the final volume to 1 L.

Step 8: Sterilize at 121° C. for 20 minutes.

This solution is crystal-clear and will remain so for long periods if kept in acid-washed glassware. It provides a complex mineral salts base with ammonium-N which has been found suitable, after addition of specific components, for a wide range of autotrophic and exacting heterotrophic bacteria. With the omission of the ammonium sulfate it is a suitable base for nitrogen-fixing bacteria. It may not be the *best* base, but in all cases tested (see below) it has been found satisfactory for taxonomic purposes.

TABLE I
Group A

Solution	Amount	Procedure
1. N NaOH	1 L.	Sterilize at 121° C. for 20 mi
2. 0.074 M H_3PO_4	1 L.	Sterilize as for Solution 1 (ab
3. Solution from 2, above	200 ml.	Dilute to 2000 ml. (0.0074 M); ilize as for Solution 1
4. Solution from 3, above	1 L.	Neutralize with use of N Na sterilize as for Solution 1
5. $NaHCO_3$ in 100 ml. of water	8.333 gm.	Sterilize as for Solution 1
6. $CaCl_2$ in 100 ml. of water	5.0 gm.	Sterilize as for Solution 1
7. $NaNO_2$ in 100 ml. of water	50.0 gm.	Sterilize as for Solution 1
8. Glucose in 100 ml. of water	10.0 gm.	Sterilize at 110° C. for 25 mi
9. Mannitol in 100 ml. of water	10.0 gm.	Sterilize as for Solution 8
10. Sucrose in 100 ml. of water	10.0 gm.	Sterilize as for Solution 8
11. Sodium citrate in 100 ml. of water	2 gm., anhydrous (or 2.77 gm., hydrated)	Sterilize at 121° C. for 20 min.
12. Phenol in 100 ml. of water	10.0 gm.	Sterilize as for Solution 11
13. $Na_2S_2O_3$ in 100 ml. of water	10.0 gm.	Sterilize as for Solution 11
14. 0.5 N HCl	100 ml.	Sterilize as Solution 11
15. 0.0167 M H_3PO_4	1 L.	Neutralize 500 ml. with N Na sterilize as for Solution 11
16. Monoethylamine hydrochloride in 100 ml. of water	5 ml.	Sterilize by filtration

TABLE II
Group B

Solution	Amount	Solvent required	Final concentr
	per 100 ml. solvent		*μg./L. mediu*
1. NaCl	3.0 gm.	0.0074 M H_3PO_4	300,000
2. $(NH_4)_2SO_4$	6.6 gm.	0.074 M H_3PO_4	660,000
3. $LiCl_2$	21.0 mg.	0.0074 M H_3PO_4	21
4. $CuSO_4 \cdot 5\ H_2O$	80.0 mg.	0.0074 M H_3PO_4	80
5. $ZnSO_4 \cdot 7\ H_2O$	106.0 mg.	0.0074 M H_3PO_4	106
6. H_3BO_4	600.0 mg.	0.0074 M H_3PO_4	600
7. $Al_2(SO_4)_3 \cdot 18\ H_2O$	123.0 mg.	0.0074 M H_3PO_4	123
8. $NiCl_2 \cdot 6\ H_2O$	110.0 mg.	0.0074 M H_3PO_4	110
9. $CoSO_4 \cdot 7\ H_2O$	109.0 mg.	0.0074 M H_3PO_4	109
10. $TiCl_4$	60.0 mg.	0.074 M H_3PO_4	60
11. KBr	30.0 mg.	Water	30
12. KI	30.0 mg.	Water	30
13. $MnCl_2 \cdot 4\ H_2O$	629.0 mg.	0.074 M H_3PO_4	629
14. $MgSO_4 \cdot 7\ H_2O$	1.4 gm.	Water	140,000
15. $SnCl_2 \cdot 2\ H_2O$	36.0 mg.	Water	36
16. $FeSO_4 \cdot 7\ H_2O$	300.0 mg.	Water	300

UM FOR NITROSOMONAS

Liquid Medium: Adjust the pH of the mineral salts medium to 8.2 before izing. Add aseptically 20 ml. of the e $NaHCO_3$ solution (Solution 5, p A) per L.

Silica Gel Plates: In the preparation e silica gel plates the medium is di- 1:2. To allow for this, prepare a le strength basal mineral salts medium B.M.S.M.) as follows.

llow the instructions for the single gth medium to *Step 2*. Add 105 ml. of led water and make the volume up to oximately 400 ml. with the use of the 7 M H_3PO_4 (Solution 15, Group A). ralize with N NaOH (Step 3). Then v Steps 4, 5, and 6 as indicated. Make inal volume to 500 ml. with the use ie neutralized 0.0167 M H_3PO_4 (Solu- 15, A) and sterilize at 121° C. for 15 ites.

epare the silicic acid by the method ramer (see Preparation of Silica Gel).

efore gels can be prepared it is neces- to determine the quantity of N NaOH ution 1, Group A) required to adjust pH to the desired level after the addi- of $NaHCO_3$ and the $CaCl_2$ solutions d 6, Group A, respectively).

a trial, mix 10 ml. of silicic acid and l. of D.S.B.M.S.M.

utralize with N NaOH and note the unt added (x). Add 0.4 ml. of the CO_3 solution (5, Group A), and then st the pH to 8.2 with N NaOH (1, up A). Divide the sample (approxi- ely 20 ml.) into four aliquots, and add ing amounts of the $CaCl_2$ solution to . Allow 2 to 3 hours to gel, and then rmine the most suitable amount of l_2 solution (y) required for the whole nl. sample (approximately 0.6 to 0.8 is required).

efore proceeding to pour the plates, are another test plate as follows. Mix:

ic acid	10 ml.
.B.M.S.M.	10 ml.
aOH (1, Group A)	x ml.
$CaCl_2$ solution (6, Group A)	y ml.
Inoculum	1 ml.
$NaHCO_3$ solution (5, Group A)	0.4 ml.

Immediately determine the pH and add N NaOH (Solution 1, A) until the pH rises to 8.2. Note this amount (x_1).

To prepare the plates substitute the value of ($x + x_1$) for x in the previous mix.

Allow 2 hours to gel and then incubate in a humid chamber at 28° C.

MEDIUM FOR NITROBACTER

1. *Liquid Medium:* Prepare the basal mineral salts medium with *omission* of the ammonium sulfate (Solution 2 of Group B) and sterilize.

Add aseptically 8 ml. of the $NaNO_2$ solution (7, Group A) and 20 ml. of the sterile $NaHCO_3$ solution (5, Group A) per L. of medium. Adjust the pH aseptically to 8.8 with sterile N NaOH (1, Group A).

2. *Silica Gels:* Proceed as for *Nitrosomonas* with *omission* of the ammonium sulfate (2, Group B) in the preparation of the double strength basal mineral salts medium (D.S.B.M.S.M.). Then mix:

Silicic acid	10 ml.
D.S.B.M.S.M	10 ml.
$NaNO_2$ solution (7, Group A)	0.16 ml.

Neutralize with N NaOH (1, Group A) noting the amount used (x). Add 0.4 ml. of the $NaHCO_3$ solution (5, Group A), and then adjust the pH to 8.8. Divide the sample into four aliquots; add varying amounts of $CaCl_2$ solution (7, Group A) to each and allow to stand for 2 to 3 hours to gel. Determine the amount of $CaCl_2$ solution (y) which will give the optimal gel in the total (approximately 20 ml.) sample.

Prepare another mix as follows:

Silicic acid	10 ml.
D.S.B.M.S.M	10 ml.
$NaNO_2$ solution (7, Group A)	0.16 ml.
Inoculum	1 ml.
N NaOH (1, Group A)	x ml.
$CaCl_2$ solution (6, Group A)	y ml.
$NaHCO_3$ solution (5, Group A)	0.4 ml.

Determine the pH immediately; adjust

to pH 8.8 with N NaOH (1, Group A) and note the amount (x_1).

To prepare plates substitute the value of ($x + x_1$) for x in the previous mix.

Allow 2 hours to set and then incubate at 28° C. in a humid chamber.

NITROGEN-FREE LIQUID MEDIUM FOR DETECTION OF NITROGEN-FIXING ORGANISMS

Prepare the basal mineral salts medium with *omission* of the ammonium sulfate (2, Group B) and substitution of 110 ml. for 210 ml. of water in Step 2. Adjust the pH to 7.0* and sterilize at 121° C. for 15 minutes. Cool and add aseptically 100 ml. of Group A Solution 8, (glucose), 9 (mannitol), or 10 (sucrose).

Note: If the latter solutions show any trace of yellow color, prepare separately 10 per cent solutions of glucose, sucrose, and mannitol and sterilize by filtration. Add aseptically 1 ml. of sugar to 10 ml. of solution.

The medium should be dispensed in layers not more than 1 cm. deep in Erlenmeyer flasks. Flasks must be incubated in a sealed container over N H_2SO_4 to remove ammonia from the air or in a chamber through which ammonia-free air is continuously passed.

LOW PH MEDIUM FOR ACIDURIC SPECIES OF *Thiobacillus*

Prepare the basal mineral salts medium. Add 10 gm. of $Na_2S_2O_3$ or 10 gm. of sulfur per L. Adjust the pH to 4.5 and sterilize at 121° C. for 20 minutes.

The medium should be dispensed in layers not more than 1 cm. deep in Erlenmeyer flasks.

NEUTRAL MEDIUM FOR NONACIDURIC SPECIES OF *Thiobacillus*

Follow the preparation of the basal medium except at Step 2 add 110 ml. of water instead of 210, and at Step 7 make the final
volume to 900 ml. instead of 1000. Ster
at 121° C. for 20 minutes. Cool. Then
aseptically 100 ml. of sterile $Na_2S_2O_3$ s
tion (13, Group A) and 20 ml. of st
$NaHCO_3$ solution (5, Group A).

The medium should be dispensed
depth of not more than 1 cm. in E
meyer flasks.

PHENOL MINERAL SALTS MEDIUM FOR *M plana*

Prepare the basal mineral salts medi
but substitute 110 ml. water for the
ml. at Step 2, and make the final vol
to 900 ml. at Step 7.

Sterilize at 121° C. for 15 minutes.

Add aseptically 100 ml. of the st
phenol solution (12, Group A). Adjust
pH, if necessary, with sterile N NaOH
Group A).

Dispense the medium in layers not
than 1 cm. deep in Erlenmeyer flasks
least three transfers should be achieved
fore growth of an organism is consid
positive.

CITRATE MINERAL SALTS MEDIUM

Prepare the basal mineral salts med
but substitute 110 ml. of water for the
ml. at Step 2 and make the final volum
900 ml. at Step 7.

Sterilize at 121° C. for 20 minutes.

Add aseptically 100 ml. of the sod
citrate solution (11, Group A) and ad
the pH aseptically to between 6.7 and
with sterile 0.5 N HCl (14, Group A).

ALKYLAMINE MEDIUM FOR *Protaminob*

Prepare the basal mineral salts med
but omit the ammonium sulfate (2, Gr
B); substitute 160 ml. of water for the
ml. at Step 2; and make the final vol
to 900 ml. at Step 7.

Sterilize at 121° C. for 20 minutes.
aseptically 100 ml. of the sterile alkyla
solution (16, Group A).

Dispense the medium aseptically
layers not more than 1 cm. deep in E
meyer flasks.

---

* *Beijerinckia* does not grow in this medium at a pH of 4.5 but grows readily at pH 6.4.

e: If monoethylamine is used it will
e neutralizing before it is added to
edium.

ROPHIC MEDIUM FOR *Hydrogenomonas*

pare the same medium as that em-
l for *Nitrosomonas*, but adjust the
7.0. Incubate under an atmosphere
per cent CO_2, 30 per cent air, and
r cent hydrogen.

TROPHIC MEDIUM FOR *Methanomonas*

the same medium as for *Hydrogeno-*
s but incubate under an atmosphere of
r cent methane and 50 per cent air.
Preparation of Gases.)

TROPHIC MEDIUM FOR *Carboxydomonas*

e the same medium as for *Hydrogeno-*
s but incubate under an atmosphere
per cent oxygen and 80 per cent car-
nonoxide. (See Preparation of Gases.)

ULOSE MINERAL SALTS MEDIUM (AGAR)

epare the *double strength* basal mineral
medium as for *Nitrosomonas*. Sterilize
1° C. for 20 minutes.

epare a 3 per cent clarified agar (p.
adjust the pH to 7.0 and sterilize at
C. for 20 minutes.

prepare the plates, melt the agar and
the agar, the double strength basal
m, and the precipitated cellulose (p.
at 60° C.

x the agar and basal medium in equal
tities and pour 10-ml. amounts into
e petri dishes. Allow to gel.

d one volume of the cellulose suspen-
aseptically to the double strength
medium and thoroughly agitate to
rse the cellulose. Mix the suspension
qual amounts with clarified agar and
in 5-ml. amounts evenly over the sur-
of the prepared gels. Allow to gel.

te: The concentration of the agar used
vary with the brand. The final gel
be just firm, otherwise the cellulose-
ting organisms are unable to reach
ellulose fibers.

Incubate the plates in a humid chamber for several weeks after inoculation.

FILTER PAPER AGAR

Prepare as for cellulose mineral salts medium (agar) above with substitution of a filter paper for the layer of cellulose agar. Double the quantity of the medium placed in the dish to allow for evaporation, and incubate in a humid chamber.

Preparation of Silicic Acid Sols

To prepare an ion exchange column take approximately a 70-cm. length of 25 mm. glass tubing and fit it at one end with a glass tap and mount it vertically in a stand. Place some glass beads in the base and cover with a layer of glass wool.

Pack 120 gm. (wet weight) of IR-120 Amberlite resin in the tube and add sufficient 2 N HCl to cover the resin. Remove any air bubbles from the glass column with a glass rod. Open the tap and pass 1000 ml. of 2 N HCl through the column. Drain and then flush with distilled water until the effluent no longer gives a test for chloride with silver nitrate. Leave the column full of water.

Immediately after using the column for the preparation of the silicic acid wash it again with water and regenerate with 2 N HCl.

Prepare 500 ml. of a solution of sodium silicate containing 1.5 per cent of SiO_2 and allow it to flow through the column at 5 ml. per minute. Check the pH of the effluent and collect for use when the pH falls below 3.4. Adjust the pH of the sol to 2.0 with HCl. The sol should be stable at this pH. Sterilize at 110° C. for 25 minutes.

Reproduced by permission from D. Pramer, Appl. Microbiol., *5*, 1957, 392.

Autotrophic Media for *Nitrosomonas* and *Nitrobacter*

NaCl	0.3 gm.
$MgSO_4 \cdot 7H_2O$	0.14 gm.
$FeSO_4 \cdot 7H_2O$	0.03 gm.

H_2O	90 ml.
0.1 M KH_2PO_4*	10 ml.
+ $(NH_4)_2SO_4$ (for *Nitrosomonas*)	0.66 gm.
+ $NaNO_2$ (for *Nitrobacter*)	0.5 gm.

Dilute to 1000 ml. and add 10 gm. of powdered $CaCO_3$ and 0.4 ml. of a trace element solution supplying Mn, 22 µg.; B, 21 µg.; Cu, 17 µg.; Zn, 16 µg.; and Co, 14 µg.

Dispense in layers not more than 1 cm. deep in Erlenmeyer flasks. Sterilize at 121° C. for 15 minutes.

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Quantitative Determination of Ammonium-N

Distill a sample of 2 ml. containing 20 to 500 µg. of ammonium-N with 1 ml. of 5 N NaOH in a Markham microdistillation apparatus. Use 2 ml. of 0.5 per cent boric acid containing 40 ml. per L. of Conway and O'Malley's indicator (bromocresol green, 0.033 per cent and methyl red, 0.066 per cent in alcohol; it keeps indefinitely).

After about 10 ml. of distillate has been collected, titrate the contents of the receiver in a stream of CO_2-free air with N/70 sulfuric acid from a 1.0-ml. burette. The end point is sharp and estimations are repeatable to within 0.010 ml. (≡ 1.0 µg. per ml. of ammonium-N in the sample).

Reproduced by permission from H. Lees and J. H. Quastel, Biochem. J., *40*, 1946, 803.

Quantitative Determination of Nitrate in Culture Media

Volumetric: If the sample to be examined contains no more than a few micrograms of nitrite this can be ignored. If the nitrite is present in large amounts remove it from the sample by adding a crystal of potassium iodide in the presence of sulfuric acid and bubbling nitrogen through the sample. Apply a spot test for nitrite before proceeding.

To 3 ml. of the sample containing 20 to 500 µg. of inorganic nitrogen in a hard glass tube, add 0.15 gm. of iron and 3 ml. per cent sulfuric acid. Place the tube beaker of water at 70° C. When the effervescence has subsided, close the tightly with a rubber bung and set it overnight. This procedure reduces al organic nitrogen compounds to amm Proceed as for the determination of monia with the use of the whole samp a measured aliquot in the Markham

The titration of the distillate is equ lent to the (nitrate + nitrite + am nium)-N in the 3-ml. sample, and from the nitrate-N can be determined by traction of the (nitrite + ammonium

Reproduced by permission from H. and J. H. Quastel, Biochem. J., *40*, 1 803.

Note: If polarographic equipment is a able, nitrate can be determined by method described by Skerman and M Rae, Can. J. Microbiol., *3*, 1957, 215.

Quantitative Determination of Nit

Dilute a sample containing appr mately 0.5 to 5.0 µg. of nitrite-N to 11 with distilled water. Add 2 ml. of Gr Llosvay reagent. After 30 minutes read color density in a photoelectric colorim with the use of a suitable filter and de mine the nitrite-N concentration fro curve prepared from a series of stand nitrite solutions.

GRIESS-LLOSVAY REAGENTS

1. Sulfanilic Acid Solution: Dissolve gm. in 30 ml. of glacial acetic acid. Add ml. of distilled water and filter. The agent is stable for 1 month.

2. α-Naphthylamine Solution: should not be more than 1 week old.

Dissolve 0.1 gm. of α-naphthylamin 100 ml. of boiling distilled water. Cool add 30 ml. of glacial acetic acid. Fil For use mix Solutions 1 and 2 in e quantities.

STANDARD NITRITE SOLUTION

Dissolve 0.493 gm. of pure sodium nit in water. Make up to 1 L. Dilute 10

---

* Previously boiled for 30 minutes, cooled, and made up to volume.

s solution to 1 L. This final solution
ns 0.001 mg. of N as nitrite. The
ard curve should be prepared over a
of 0.0005 and 0.001 mg. of nitrite
en.

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and J. H. Quastel, Biochem. J.,
46, 803.

Reduction of Nitrate to Nitrite

e: This reaction is dependent on a
er of factors. 1. It obviously will not
if the medium in which the nitrate
orporated does not support the nor-
rowth of the organism concerned. The
eed not be restricted to the use of the
ne basal medium normally employed,
or comparative purposes the behavior
e organism in the nitrate-peptone
r should be recorded.

It will not occur if the organism re-
s a supply of oxygen adequate for all
rational needs during growth as may
r in (*a*) still cultures of slow-growing
in which the medium is distributed in
ow layers which admit oxygen by dif-
n as rapidly as it is utilized by the cells,
) in aerated cultures. This is due to one
wo factors. The nitrate-reducing en-
is not formed in the presence of an
uate oxygen supply and the enzyme,
formed, does not function under such
itions.

ere are reports of strictly aerobic auto-
ic organisms which will use nitrate
e sole source of nitrogen; this suggests
reduction is occurring in the presence
xygen. In view of the generally slow
th of such organisms and the presence
dequate amounts of ammonia in the
age laboratory air, such reports must
onsidered with caution. It is possible
some intracellular mechanism is in-
ed in the reduction of nitrate for syn-
ic purposes.

nder anaerobic conditions, such as
in deep still cultures incubated aer-
ally after the dissolved oxygen is uti-
by the growing cells *and* under strictly anaerobic conditions, nitrate may be reduced to nitrite, ammonia, nitrous oxide, or gaseous nitrogen.

With organisms in which the reduction is limited to nitrite the latter is readily detected. With the others the detection of nitrite will depend on the degree to which it accumulates at any time during growth and the actual time at which the test is performed. For this reason simple testing of the medium for nitrite at a fixed time interval after inoculation is valid only if the test is positive. A negative reaction is inconclusive.

The use of suspensions of cells grown anaerobically in the presence of nitrate to adapt them to nitrate reduction, in tests employing a substrate and nitrate is also subject to error, since in the presence of certain substrates some organisms will reduce nitrate to nitrogen without any detectable production of nitrite.

PROCEDURE

Select the medium most suited to the growth of the organism. Incorporate in the medium 0.2 per cent KNO_3, dispense in 5-ml. amounts in 150- by 13-mm. tubes, and sterilize. Allow the tubes to cool in air for 24 hours to permit the oxygen in the medium to equilibrate with air (except where required for anaerobic cultures). Inoculate the medium and cover it with a sterile paraffin seal. Incubate in a water bath at the optimal temperature. Observe the development of turbidity in the medium, and begin spot tests for nitrite as soon as the medium becomes obviously turbid to the naked eye. At this stage there is rarely any dissolved oxygen left in the medium. Remove the test samples with a warmed sterile dropping pipette, and reseal the paraffin after the removal of the sample. Continue the tests until maximal turbidity has been reached and for 12 hours beyond this time.

If the nitrite test is at any stage positive, record as "nitrite produced from nitrate" and specify the medium. If the latter is not nitrate-peptone water, the comparative reaction in nitrate-peptone water should be included and recorded even if no growth occurs.

If nitrite tests are continually negative and there is no evidence of gas production, it is unlikely that nitrate is reduced.

Gas production may be due to evolution of nitrogen or nitrous oxide. The nature of the gas has not, as yet, any taxonomic significance. It should be remembered, however, that some organisms produce gas from peptone water alone under anaerobic conditions so that nitrate-free controls should be used.

1. THE STARCH-IODIDE SPOT TEST FOR NITRITE

Reagents: Starch Iodide Solution

Starch	0.4 gm.
$ZnCl_2$	2.0 gm.
H_2O	100 ml.

Dissolve the $ZnCl_2$ in 10 ml. of water. Boil and add the starch. Dilute to 100 ml., allow to stand for 1 week, and filter. Add an equal volume of a 0.2 per cent solution of KI.

Hydrochloric Acid

Concentrate HCl	16 ml.
Water	84 ml.

Test

Using clean glass dropping pipettes, place 1 drop of each reagent in the depression of a white spot test plate. Add 1 drop of the culture. A blue color indicates the presence of nitrite.

The test depends on the formation of nitrous acid and its subsequent reaction with potassium iodide with the liberation of iodine which turns the starch blue. The test is not entirely specific. Control tests should be made with uninoculated media. Avoid the use of metal implements in taking samples.

2. THE SULFANILIC ACID-α-NAP AMINE METHOD

Reagents

See under Quantitative Determi of Nitrite.

Spot Test

To 1 drop of the mixture of solut and 2, add 1 drop of the culture. T velopment of a pink color indicat presence of nitrite.

Reference: The general procedures author's recommendation.

Autotrophic Medium for *Hydro* *monas*

KH_2PO_4	0
NH_4NO_3	0
$MgSO_4 \cdot 7\ H_2O$	0.0
$FeSO_4 \cdot 7\ H_2O$	0.00
$CaCl_2 \cdot 2\ H_2O$	0.00

Distilled water to 100 ml.

Adjust the pH to between 6.8 an Where desired, incorporate 1.5 pe washed agar. For autotrophic growth plement the base with 0.05 per $NaHCO_3$. Autoclave stock solutions $NaHCO_3$ separately, flush with CO_2 add to the sterile medium before in tion.

Incubate under an atmosphere of cent CO_2, 30 per cent air, and 60 pe hydrogen.

Reproduced by permission fro Schatz and C. Bovell, J. Bacterio 1952, 87.

Medium for the Cultivation of *boxydomonas*

KNO_3	2.
K_2HPO_4	1.0
$MgSO_4 \cdot 7\ H_2O$	0.1
Peptone	0.
H_2O	10

Dissolve the ingredients and adju pH to 7.2. Sterilize at 121° C. for 2 utes.

n agar medium use only sufficient make a moderately firm gel. An ich is too hard inhibits growth.

ate under an atmosphere of 80 per and 20 per cent O_2.

nce: A. Kistner, Proc. Koninkl. ad. Wetenschap., Ser. C. *56*, 443,

phic Medium for *Methano-*

	2.0 gm.
7 H_2O	0.2 gm.
H_2O	0.001 gm.
$_4$	0.21 gm.
$_4$	0.09 gm.
5 H_2O	200.0 μg.
	60.0 μg.
·H_2O	30.0 μg.
H_2O	300.0 μg.
	15.0 μg.
	0.04 gm.
	0.015 gm.
	1000 ml.

lve the salts and sterilize. Incubate n atmosphere of 50 per cent meth- 50 per cent air.

roduced by permission from J. W. (personal communication).

A Simple Apparatus for Observing the Oxidation of Carbon Monoxide, Hydrogen, or Methane by Growing Cultures

The apparatus is illustrated in figure 1. *A* consists of a 10-ml. graduated pipette sealed at the tip and joined above the 0.0-ml. mark to a 12-ml. tube fitted with a 12-mm. side arm and pear-shaped bulb with a 16-mm. outlet. The tube is plugged at the outlet with cotton wool and sterilized.

B is a glass rod of 6-mm. outside diameter, bent so that the external arm lies parallel to the main axis of the tube when the rod is inserted as illustrated. The external end is unconstricted and is plugged with cotton wool. The internal end is only slightly constricted and extends into the tube until it almost touches the wall when the rubber stopper *D* fits snugly into the base of the side arm. A small 1-mm. aperture, *E*, is made in the tube exterior to the rubber stopper.

A cotton wool plug is rolled around the stem of *B* between the rubber stopper and the bend. The lower end (bearing the stopper) is inserted into a 150- by 16-mm. tube; and the assembly sterilized.

A third tube, *C*, bent in the same manner as *B* but without the small hole *E* and

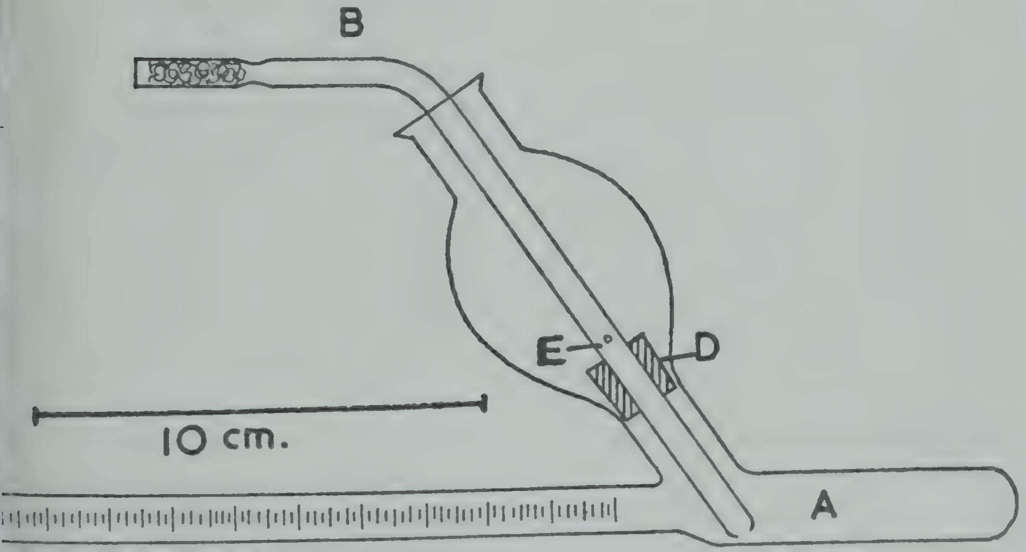

Fig. 1

with the external end extended to a length which brings its tip level with the top of the graduated tube, completes the apparatus. This tube is fitted with a rubber stopper similar to *D*.

Method for Use: Pipette 40 ml. of the sterile synthetic medium into the tube *A* and inoculate the medium. Replace the plug in *A* with tube *B*, leaving the stopper *D* only loosely seated in its base.

Connect *B* to the gas-mixing burette and, holding the tube on its side, loosen the stopper *D* and allow a quantity of gas to escape into the pear-shaped bulb to exclude the air in tube *B*. Then turn the tube upright and collect approximately 9 ml. of gas. If too much is collected it can be released by inclining the tube. Disconnect the gas supply and seat *D* firmly into the side arm. Supporting the tubes by the outlet tube, immerse them for 20 minutes in a water bath at a temperature as near that of the room as possible. *Holding the tubes by the outlet tubes,* slightly tilt the tube until the menisci in the closed arm and pear-shaped bulb are level; read the gas volume (V_1) and note at the same time the temperature of the water bath. *All subsequent gas volume readings must be taken at the same temperature.* Incubate the tubes on their sides attached to a rocking arm in a water bath. This provides a maximal gas-liquid contact area during incubation. The small aperture *E* allows movement of liquid between the two bulbs but the position of the internal end of *B* does not allow escape of gas.

After incubation adjust to room temperature to eliminate errors due to gas expansion. Replace tube *B* with tube *C*. Seat the stopper well into the side arm and introduce water through *C*, while holding the tube in the vertical position by the outlet tube, until the levels in the closed arm and in *C* are equal. Read the gas volume (V_2).

Release the stopper in the side arm and insert 4 pellets of solid sodium hydroxide (approximately 0.4 gm.) into the closed tube. Seal the tube and slowly rock it on its side in a water bath at room temp
ture for 20 minutes to absorb CO_2 anc
equilibrate the temperature. Place i
vertical position, readjust the le
through *C*, and again read the volume (
The difference ($V_2 - V_3$) is CO_2.

Release *C* and introduce 0.5 gm. of s
pyrogallic acid by allowing it to c
through into the closed arm. Reseal;
the tubes to absorb the oxygen; equilib
the temperature; adjust the levels;
read the volume (V_4). The difference V
V_4 is oxygen. The residual gas will be
drogen, carbon monoxide, or methane,
pending upon the mixture used for cult

If the final gas analysis is compared
the initial one the ratio of gas oxidize
oxygen utilized can be determined.

Reference: Author's recommendatio

Preparation of Gases

CARBON MONOXIDE FOR GROWTH OF *boxydomonas*

Close a round-bottomed 500-ml.
with a rubber stopper fitted with a
outlet tube just penetrating the stopper
a dropping funnel with the lower end re
ing almost to the bottom of the flask.
concentrated sulfuric acid to the flas
that the tip of the dropping funnel is
mersed. Place concentrated formic aci
approximately half this volume in the d
ping funnel. Place the flask over a st
bath and connect the gas outlet tube
a concentrated NaOH wash bottle or s
lime tube to the gas-collecting appara

Heat to 100° C. and then admit the
centrated formic acid drop by drop
the required amount of gas has been
lected. Allow for the exclusion of air b
collecting the gas.

$$HCOOH + H_2SO_4 \rightarrow H_2SO_4H_2O + C$$

Reproduced by permission from G
Parkes and J. W. Mellor, Mellor's Mo
Inorganic Chemistry, Longmans, G
and Company, New York. p. 359. 1947

GEN

apparatus similar to that employed
preparation of carbon monoxide but
ate the wash bottle.
e granulated zinc in the flask and
with water until the lower end of the
ing funnel is immersed. Add concen-
sulfuric acid dropwise from the drop-
nnel. Allow a volume of gas equal to
st twice the volume of the flask to
before collecting over water in a gas
e.

N DIOXIDE

pare the CO_2 in the same way as
gen, but substitute cracked marble
e granulated zinc and concentrated
chloric acid for the sulfuric acid. Col-
ver dilute sulfuric acid.

EN

dically pure oxygen is so widely avail-
that the preparation of oxygen is
ly unnecessary. It may be prepared as
s.
x equal quantities of potassium chlo-
nd *carbon-free* manganese dioxide in
e-mouthed Florence flask and connect
wide delivery tube, through a soda-
tube, to the gas-collecting burette.
the mixture carefully over a flame
the gas is evolved. CO_2 and chlorine
moved in the soda-lime tube.

ANE

e methane may be prepared by re-
g methyl iodide in alcohol with a cop-
nc couple.
100 gm. of zinc dust to 250 ml. of a
cent aqueous solution of copper sul-
Shake thoroughly and then allow the
er to settle. Wash several times with
by decantation and then dry.
ce the powder in a 100-ml. Erlen-
r flask fitted with a stoppered drop-
funnel and a gas outlet tube. Allow
ture of equal volumes of methyl io-
and absolute ethyl alcohol to drop
onto the zinc copper couple. Collect the methane over water after allowing the air from the flask to escape.

Reproduced by permission from C. Weygand, Organic Preparations, Interscience Publishers, New York, 1945, p. 41.

Apparatus for the Collection and Mixing of Gases

In culturing autotrophic gas-utilizing organisms it is necessary to prepare gas mixtures of various types. This can be done simply with the apparatus shown in figure 2. This consists of a series of 500-ml. gas-collecting burettes *A*, *B*, *C*, and *D*, connected at the base via a manifold to a 3-L. reservoir, *E*, containing dilute sulfuric acid, and via taps at the top to a second manifold closed at both ends by taps *F* and *G*. The tube from tap *G* is connected to the gas generator and both *G* and *F* are opened. Gas is allowed to stream through *G* and *F* to expel air or any previous gas. Then with *F* still open, the tap to *A* is opened and then *F* is closed. The gas is diverted into *A* and dilute acid is expelled to *E*. If desired *E* may be lowered to reduce the back pressure on the gas generator. With *A* nearly filled, open *F* and then close *A* immediately. Disconnect the gas generator and then close *G* and *F*. Repeat with other gas to *B* and *C*. Any number of gas burettes may be employed but allowance must be made for mixing.

To prepare a mixture of 200 ml. of *A*, 100 ml. of *B*, and 200 ml. of *C* and *D*, open *F* and then *A* to flush out the manifold. Close *F*. Lower *E* so that its meniscus is level with the 200-ml. mark in *D*. Open *D* and let the gas flow in, adjusting *E* so that its meniscus is finally level with the water in *D* at the 200-ml. mark. Close *D* and *A*. Raise *E* and open *B* and *F* to flush the manifold. Close *F*. Lower *E* to the 300-ml. level of *D* and open *D*. Allow gas from *B* to flow into *D* to the required level. Close *D* and *B*. Raise *E* and open *C* and *F* to flush the manifold. Close *F*. Lower *E* to the 500-ml. mark in *D* and open *D*.

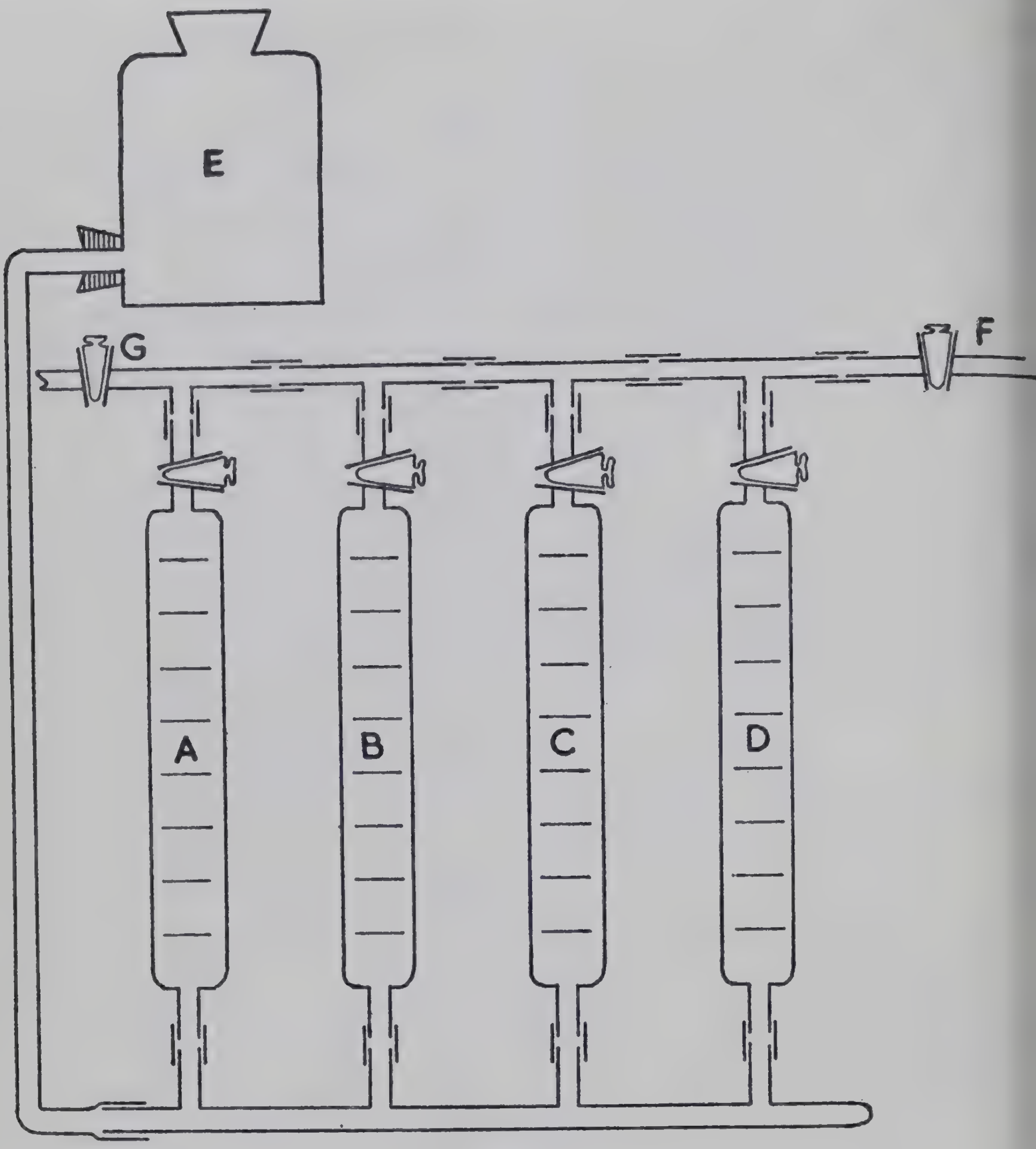

FIG. 2

Allow gas from *C* to flow in to the required level. Close *D* and *C* and replace *F*.

To discharge *D* into the required containers, open *G* and *D* and flush the manifold. Close *G*. Connect *F* to the apparatus and open *F*. Allow some of the gas mixture to flow through the connection to the apparatus to expel any air before admitting the gas.

References: Author's recommendation.

Autotrophic Medium for Aciduric Species of *Thiobacillus*

NH_4Cl	0.1 gm.
KH_2PO_4	3.0 gm.
$MgCl_2 \cdot 6\ H_2O$	0.1
$CaCl_2$	0.1
$Na_2S_2O_3 \cdot 5\ H_2O$	5.0
Or sulfur	10.0
Distilled water	1000

Adjust the pH to 4.2. If thiosulfa used steam for 1 hour on 3 successive If sulfur is used, sterilize the rest of medium at 121° C. for 20 minutes, steam the sulfur in a separate tube add aseptically.

Reproduced by permission from C Parker and J. Frisk, J. Gen. Microbio 1953, 344.

rophic Medium for Nonaciduric s of *Thiobacillus*

$_2SO_4$	0.1 gm.
O_4	4.0 gm.
O_4	4.0 gm.
$_4 \cdot 7\ H_2O$	0.1 gm.
	0.1 gm.
$6\ H_2O$	0.02 gm.
$_4 \cdot 4\ H_2O$	0.02 gm.
$O_3 \cdot 5\ H_2O$	10 gm.
ed water	1000 ml.

ust the pH to 6.6, and steam for 1 n 3 successive days.

roduced by permission from C. D. r and J. Frisk, J. Gen. Microbiol., 3, 344.

trophic Medium for *Thiobacillus* *xidans*

$\cdot 7\ H_2O$	130.0 gm.
$_4 \cdot 7\ H_2O$	1.0 gm.
$_2SO_4$	0.5 gm.
led water	1000 ml.

solve the ingredients and adjust the between 2.0 and 2.5 with sulfuric Autoclave at 121° C. for 15 minutes llow to stand. A voluminous precipi- f ferric hydroxide settles out. Remove upernatant aseptically and distribute uired, preferably in layers not more cm. deep in Erlenmeyer flasks.

prepare an agar medium dissolve the s sulfate in 300 ml. of the water and ze separately. Dissolve the other lients plus 20 gm. of agar in the ning water and sterilize. Mix the two ons just before plate pouring.

roduced by permission from K. L. le and A. R. Colmer, J. Bacteriol., 51, 605.

trophic Medium for *Ferrobacillus*

D MEDIUM

$)_2SO_4$	0.15 gm.
	0.05 gm.
$_4 \cdot 7\ H_2O$	0.50 gm.
O_4	0.05 gm.
$O_3)_2$	0.01 gm.

solve the salts in 1000 ml. of distilled water. Sterilize at 121° C. for 15 minutes. Prepare a stock solution consisting of 10 per cent $FeSO_4 \cdot 7\ H_2O$ in distilled water and sterilize by filtration; add 1 ml. aseptically to each 100 ml. of the above medium. The resultant medium is opalescent. Refrigerate the medium so that oxidation does not occur.

SOLID MEDIUM

This may be prepared with silicic acid. The inorganic solution for such a solid medium is prepared as follows.

$(NH_4)_2SO_4$	6.0 gm.
KCl	0.05 gm.
$MgSO_4 \cdot 7\ H_2O$	0.50 gm.
$Ca(NO_3)_2$	0.01 gm.

Dissolve the salts in 250 ml. of distilled water. Dispense in 25-ml. aliquots in 125-ml. Erlenmeyer flasks and sterilize for 15 minutes at 121° C. Sterilize a buffer solution consisting of 13.5 gm. of K_2HPO_4 in 100 ml. of distilled water separately. Prepare the ferrous sulfate solution as for the liquid medium.

Prepare the silicic acid sol (p. 147).

Add 1 ml. of ferrous sulfate solution to 75 ml. of the silicic acid sol and 1 ml. of the sterile phosphate buffer to 25 ml. of the sterile salts solution. Mix the solutions and allow to set for 24 hours.

Reproduce by permission from W. Leathen, N. A. Kinsel, and S. A. Braley, Sr., J. Bacteriol., *72*, 1956, 700.

Phenol Mineral Salts Medium for *Mycoplana*

The original medium described by Gray and Thornton is prepared as follows.

K_2HPO_4	1 gm.
$MgSO_4 \cdot 7\ H_2O$	0.2 gm.
NaCl	0.1 gm.
$CaCl_2 \cdot 2\ H_2O$	0.1 gm.
$FeCl_3$	0.02 gm.
$(NH_4)_2SO_4$	0.5 to 1.0 gm.
Or KNO_3	0.5 to 1.0 gm.
Phenol	10 gm.

Dissolve the ingredients, adjust the pH

to 7.0, and sterilize at 121° C. for 20 minutes.

Serial transfers of the organisms must be achieved before growth in this medium is recorded as positive.

NOTE: The above medium yields a precipitate which is not desirable when turbidity is used as an index of growth. An equally satisfactory medium which is free of precipitate is described under Pope-Skerman Mineral Salts Media for the Cultivation of Autotrophs and Nonexacting Heterotrophs.

Reproduced by permission from P. H. Gray and H. G. Thornton, Zentr. Bakteriol. Parasitenk., Abt. II, *73*, 1928, 74.

Alkylamine Media for the Identification of *Protaminobacter*

The original medium employed by den Dooren de Jong is prepared as follows.

$MgCl_2$	2.0 gm.
K_2HPO_4	10.0 gm.
Tap water	1000 ml.
Washed agar	14 gm.

Dissolve the ingredients, filter, and adjust the pH to 7.0. Distribute in 20-ml. quantities and sterilize at 121° C. for 20 minutes.

Prepare a 5 per cent solution of the alkylamine. Neutralize with HCl. (Either monoethylamine or dimethylamine satisfies the requirements of the described species.*) Sterilize by filtration and store in sterile screw-capped bottles or glass ampoules.

To prepare the medium, melt the agar base and cool to 50° C. Add 1 ml. of the alkylamine solution to 20 ml. of the agar base and pour.

NOTE: Since the majority of agars available commercially contain quantities of metabolizable materials, growth of organisms on the above medium is not an indication of use of the alkylamine. A liquid medium prepared with glass-distilled water should be employed as a final check on the ability of the organism to grow with the of the alkylamine as sole carbon nitrogen sources.

A liquid medium prepared by the ab formula is turbid. An equally satisfact growth medium which is free of precipit is described under Pope-Skerman Min Salts Media for the Cultivation of Au trophs and Nonexacting heterotrophs.

Reproduced by permission from Dooren de Jong, Zentr. Bakteriol. Pa sitenk., Abt. II, *71*, 1927, 218.

Koser's Citrate Medium

Organisms capable of utilizing citr as the sole source of carbon will grow in following medium.

NaCl	5
$MgSO_4 \cdot 7\ H_2O$	0.2
$NH_4H_2PO_4$	1
Sodium citrate	2
K_2HPO_4	1
Distilled water	1000

Dissolve the salts in the water, adj the pH to between 6.7 and 6.9. Fil dispense, and sterilize at 121° C. for minutes.

Inoculate from an agar culture and m at least three serial transfers before re tering a definite positive result.

Reference: S. A. Koser, J. Bacteriol. 1924, 59.

Cellulose Strip-Peptone Medium

Peptone	5.0
NaCl	5.0
Tap water	1000

Steam until dissolved. Adjust the pH 7.4 and filter if necessary. Dispense in ml. amounts in 150- by 13-mm. tubes.

In each tube place a 70- by 10-mm. s of Whatman No. 1 filter paper. Steri at 121° C. for 15 minutes.

Preparation of Precipitated Cellulo

Carefully add 100 ml. of concentra sulfuric acid to 60 ml. of water in a fl cooled by running water, with care direct the neck of the flask away from

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* If the hydrochlorides of the alkylamines are used they do not need neutralization.

Cool to 70° C. and to a 5-ml. sample
3 gm. of Whatman No. 1 filter paper.
paper dissolves rapidly and subse-
y chars, proceed as follows (if the
does not dissolve prepare a slightly
concentrated acid).

e a series of ten 150- by 18-mm.
in a rack in a water bath at 70° C.
hese tubes pipette 0.2, 0.4, 0.6, 0.8,
.2, 1.4, 1.6, 1.8, and 2.0 ml. of water,
tively. To each add 10 ml. of the
d sulfuric acid and mix. To each tube
.3 gm. of filter paper (torn into small
). In the higher acid concentrations
aper usually dissolves and chars. In
wer concentrations it will disintegrate
ay not char. When dispersed in water
lisintegrated paper yields a mass of
bers which scintillate in reflected light
ettle out rapidly on standing. These
are too coarse for a good cellulose
m. At an intermediate acid concen-
n the paper is reduced to a gelatinous
which slowly turns brown if allowed
nd. If this is rapidly diluted a mass
ely divided particles is produced which
ins suspended for some period and
not scintillate in reflected light. This
rial is suitable for the preparation of
itated cellulose agar.

te the degree of dilution of the acid
lilute the bulk accordingly. Hold at
and add 3 gm. of Whatman No. 1
paper (torn in small pieces) to each
l. of acid. Rotate the flask until slight
ving (charring) is evident and then
y the contents rapidly into a 5-l. flask
ining 2000 ml. of tap water. The
ing opalescent suspension should
in dispersed for some time and show
vidence of scintillation in reflected
. Allow this to stand until the cellulose
es and siphon off the supernatant.
rifuge the deposit and resuspend in
to wash it free of acid. Repeat the
ss several times until 10 ml. of the
nsion will no longer produce an acid
ion after the addition of 1 ml. of 0.1
OH.

Titrate a sample with 0.1 N NaOH and then neutralize the bulk.

Centrifuge the cellulose and then resuspend in 20 ml. of water. Progressively dilute a 1-ml. sample until 5 ml. of the diluted sample dispensed in a 10-cm. petri dish still gives a perceptible opalescence. Note the degree of dilution and dilute the bulk of the cellulose by *half* this amount. (It is further diluted in the preparation of the medium.)

Sterilize at 121° C. for 20 minutes.

Reference: Author's recommendation.

Oxidation of Cellulose to Oxycellulose

This test was used by Winogradsky for differentiation of the genera *Cellfalcicula* and *Cellvibrio*. He claimed that these organisms grow on cellulose without the production of reducing substances and rarely with the production of any visible change in the paper other than the production of a pigmented and sometimes mucoid growth. No clearing of the medium occurs when the organisms are cultured on precipitated cellulose (hydrocellulose) agar.

Winogradsky (Ann. inst. Pasteur, *43*, 1929, 549) based his claim for the "oxidation of cellulose to oxycellulose" on the following: (*a*) increased solubility of the cellulose when heated in 2 per cent NaOH at 120° C. for 30 minutes; (*b*) production of a yellow to brown color when the paper was heated to 100° C. in 1 per cent NaOH; and (*c*) increased intensity of staining with basic dyes such as methylene blue. These properties are similar to those given by chemically produced oxycellulose of the acidic type.

Although the utilization of cellulose by these organisms is not questioned, Fåhraeus (1947) has discussed the validity of the above reactions as evidence of the production of "oxycellulose" and claims that the materials giving the above reactions are polysaccharides synthesized by the developing organisms. He quotes the following observations of Norman and Bartholomew (Proc. Soil Sci. Soc. Am., *5*, 1940, 242):

"(a) The mucilage is closely associated with the bacterial cells.

"(b) Any oxidative process would give rise not only to short-chain soluble fragments but also to long-chain insoluble material which should be easily detectable. On analysis it was, however, found that the residual cellulose was not more oxidized than initially.

"(c) The presence of pentose units in an oxycellulose from cotton is impossible, but the furfural yield in the determinations exceeded that which would be expected on the basis of uronic units present, and implies that some pentose groups must be involved.

"(d) Oxycellulose could only be formed by an extra-cellular oxidative process, which would be unique, if the cell was intact, and no energy would be derived by the cell until chain-splitting had taken place to such an extent that the fragments were water-soluble and therefore could pass into the cell."

Fåhraeus further states "It is true that some of the above reasons are open to criticism,* but on the whole they seem to be sufficient for rejecting Winogradsky's theory of oxidative decomposition.

For utilization of cellulose the following method of Fåhraeus is suggested.

Filter the liquid culture medium through a Jena 1G3 sintered glass crucible under suction, retaining the cellulose. Fill the crucible with 12 per cent HCl and stir. Suck off the liquid.

Treat the cellulose in the same manner with the following in the sequence indicated: (*a*) water, (*b*) 5 per cent ammonia, (*c*) water, (*d*) alcohol (twice), and (*e*) ether. After treatment with ether, suck the cellulose dry.

Dry at 50° C. for 1 hour and at 103° for 1 hour. Allow to cool in a desicca over granulated $CaCl_2$ or silica gel a weigh.

References: G. Fåhraeus, Symbo Botan. Upsalienses, *9*. 1947, 1.

Bacteriochlorophyll

Examine a dense suspension of the org isms with a calibrated hand spectrosco Photosynthetic organisms containing b teriochlorophyll show an absorption ba at 590 $m\mu$. This band is absent, both fr the cells and the extract after extracti with 85 per cent ethyl alcohol, but a sec band appears in the extract at 774 $m\mu$ indicated by a spectrophotometer.

Chlorobium Chlorophyll

The green cells of *Chlorobium* lack absorption bands of plant chloroph at 635 to 650 $m\mu$ and of bacteriochloroph at 590 $m\mu$. Examination with a spect photometer reveals an absorption peak approximately 750 $m\mu$.

Carotenoid Pigments

These pigments are yellow, orange, r or brown. They are insoluble in water a alcohol but dissolve in chloroform, et or other fat solvents. They give a deep b color with concentrated sulfuric acid antimony trichloride.

Extract a heavy suspension of the with the following solution to rem chlorophyll pigments (if any).

Saturated KOH	20
Ethanol, 40%	40
Tap water	60

Wash the extracted cells thoroughly water, centrifuge, and transfer some of packed cells to a slide. Blot dry and a drop of sulfuric acid. If carotenoids present the cells will turn blue.

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* The authors [Norman and Bartholomew] do not accept an oxidative decomposition because intermediates are not to be found; but no hydrolysis products had been established either."

E: In most instances a blue color
n can be obtained by application of
lfuric acid directly to the dense mass
xtracted cells. Red organisms which
n prodigiosin but no carotenoids do
rn blue.

ction and Identification of Pro- sin in the Genus *Serratia*

ect examination of a heavy suspen-
f *Serratia* with a hand spectroscope
eveal two absorption bands at 490 to
μ and 540 to 560 mμ.

sh the growth from three heavily
lated plates with approximately 75
of sterile saline. Add 7.5 gm. of
H pellets slowly with continuous
ng. The color changes from pink to
e. After 2 hours add 96 per cent
ol and light petroleum (b.p. 40 to
.) in amounts approximately equal
e volume of the solution (75 ml. of
. Shake the mixture thoroughly and
off the lower alcohol-saline layer and
tract it with further small quantities
troleum ether until no more color is
ved.

ix the petroleum fractions, and extract
pigment with repeated additions of
er cent v/v ethyl alcohol containing
cent glacial acetic acid. Upon transfer
e alcohol the pink color returns.

lute the alcoholic extract with an
l amount of distilled water and add
ous 2 N NaOH until the color changes
ange.

xtract repeatedly with chloroform and
evaporate the latter to dryness *in*
with slight warming. Dissolve the
ue in 5 ml. of 85 per cent ethyl alcohol
determine the absorption in a spectro-
tometer. In this acid solution an ab-
tion peak is produced at approximately
mμ.

o similar peak is obtained after identi-
extraction methods are applied to
tosynthetic bacteria or bacteria con-
ing carotenoid pigments (author's
note). Prodigiosin does not give a blue color with sulfuric acid.

Reproduced by permission from R. Hubbard and C. Rimington, Biochem. J., *46*, 1950, 220.

Isolation and Growth of *Chromatium*

MEDIUM FOR ISOLATION

The complete medium used for this purpose cannot be subjected to autoclave temperatures because of the incompatibility of some of the constituents at high temperatures. A basal medium is prepared to which sterile solutions of other substances are added after the medium has cooled.

The solution of sodium sulfide acts both as a source of the HS⁻ and as a deoxygenating agent. Unless it is kept in a tightly sealed container in the absence of oxygen it should always be prepared before use.

Basal Medium

NH_4Cl	0.1 gm.
KH_2PO_4	0.1 gm.
$MgCl_2$	0.05 gm.
NaCl (for marine species)	30.0 gm.
Agar	20.0 gm.
Tap water	925.0 ml.

Dissolve the mineral salts in the tap water; add the agar and then heat to 121° C. for 15 minutes to dissolve the agar. Distribute in the required quantities in test tubes or screw-capped bottles. Sterilize at 121° C. for 15 minutes. For a *liquid medium* omit the agar.

Sterile Sodium Bicarbonate

$NaHCO_3$	5 gm.
H_2O	100 ml.

Sterilize at 121° C. for 15 minutes.

Sterile Sodium Sulfide

$Na_2S \cdot 9\ H_2O$	5 gm.
H_2O	100 ml.

Sterilize at 121° C. for 15 minutes.

For use of the *complete medium* melt the required number of tubes of basal medium and cool to 45° C. To each 10-ml. amount of basal medium add aseptically 0.4 ml. of $NaHCO_3$ solution and 0.2 ml. of $Na_2S \cdot 9 H_2O$ solution.

ISOLATION

To obtain crude cultures of *Chromatium* species place a layer of marine mud mixed with decaying algae in the base of a wide mouth glass jar, cover with sea water, and expose to light. The reddish growth which appears on the wall of the container nearest the light source usually contains *Chromatium.* Its growth is dependent on the reduction of sulfate in the mud and the consequent liberation of H_2S.

Pure Cultures

Two procedures may be adopted.

1. Deep Tube Isolation: This method has been used successfully by van Niel and others.

Emulsify a small amount of the reddish growth (checked microscopically for the morphological type) in a quantity of complete sterile liquid medium.

Prepare a series of melted deeps of the complete medium and hold at 45° C. Make serial dilutions of the *Chromatium* suspension in these tubes and then allow the tubes to set.

Cover each tube to a depth of 2 cm. with a 50:50 mixture of paraffin wax and liquid paraffin sterilized by heating to 160° C. for 1 hour.

Incubate at 25° to 28° C. with exposure to light. Well isolated reddish colonies appear in the higher dilutions after a period of 3 to 7 days. Select a suitable tube, sterilize the outer surface, cut the glass, remove the tube and then aseptically dissect out the colony in a sterile petri dish. Emulsify the colony in sterile complete medium and repeat the process of serial dilutions to ensure purity.

Pure cultures can be maintained through subculture in these agar deeps at 2-month intervals.

2. Agar Streak Isolation: This technique is that normally employed for surface inoculation in petri dish cultures. It been employed quite successfully in t laboratory. Incubate the inoculated pe dish anaerobically. Expose the plate light to 25° to 28° C. for 4 to 7 days. Pi off typical colonies, check for purity replating, and maintain in deeps as in *1.*

Reference: These directions were tak from the formulary of the Hopkins Mari Station, Pacific Grove, California, whe they were attributed to Helge Larsen.

Isolation and Growth of *Rhodosp rillum*

ENRICHMENT MEDIUM

NH_4Cl	1.0 g
K_2HPO_4	0.5 g
$MgCl_2$	0.2 g
NaCl	2.0 g
Yeast extract	0.1 g
H_2O	900 m

Dissolve and sterilize at 121° C. for minutes.

Prepare separately and sterilize by filtr tion: (*a*) 5.0 gm. of $NaHCO_3$ and 50.0 m of H_2O, (*b*) ethyl or amyl alcohol or 4 p cent alanine, and (*c*) 0.1 N H_3PO_4.

Add the sterile sodium bicarbonate sol tion and 1.5 to 2.0 gm. of either steri ethyl or amyl alcohol *or* 50 ml. of 4 p cent alanine. Adjust the pH to 7 wi sterile 0.1 N H_3PO_4.

Enrichment: The most suitable inocul is mud in which there is a large amount organic matter undergoing decompositi in a position exposed to light.

Place a layer of the inoculum in a 50-m ground-glass-stoppered bottle, fill the bot to capacity with the medium, and ins the stopper so as to exclude air. Incuba in an illuminated cabinet at 30° C. w the vessels located approximately 4 inc from a 25-watt tungsten filament lamp.

A reddish growth usually appears in days in the solution and the mud on t side of the vessel exposed to light. Of t three substrates suggested ethyl alcoh and alanine are the most suitable.

Harvest 1 ml. of this enriched mater

larly from the reddish area, into a l bottle of the medium. Development red *Rhodospirillum* will usually occur ays.

TION MEDIUM

the enrichment medium, anaerobic ions are rapidly established by con- ating organisms. For pure cultures genation is effected by the addition $_2S\cdot 9\ H_2O$. To improve growth the ntration of yeast extract is increased 0.01 to 0.2 per cent. To solidify the um 1.5 to 2.0 per cent agar is added. pare the medium as for the enrich- but substitute 2.0 gm. of yeast ex- for the 0.1 gm. in the original medium reduce the amount of sodium bicar- te from 5.0 gm. to 2.0.

epare separately a solution containing m. of $Na_2S\cdot 9\ H_2O$ in 10 ml. of water terilize at 121° C. for 15 minutes. Add solution to the medium before finally sting the pH. Dispense as desired.

lation: The organisms may be isolated he shake tube method or by pour ng. In the former, anaerobic conditions e rapidly established in the agar deeps result of removal of oxygen by the so- sulfide.

pour plates are used the anaerobic od involving pyrogallic acid and m carbonate is recommended.

produced by permission from C. B. Niel, Bacteriol. Revs., *8*, 1944, 1.

tion and Growth of *Rhodomicro-*

suppress contaminants the medium for the isolation of *Rhodomicrobium* ore restrictive than that used for its sequent maintenance.

NTENANCE MEDIUM

st Extract-Mineral Salts Base

st extract	2.0 gm.
l	2.0 gm.
$_4)_2SO_4$	1.0 gm.
PO_4	0.5 gm.
$MgSO_4\cdot 7\ H_2O$	0.1 gm.
Water	700 ml.
Agar (if required)	15 gm.

Dissolve the ingredients by steaming, filter if necessary, and sterilize at 121° C. for 15 minutes.

Sterile Sodium Bicarbonate

Dissolve 5 gm. of $NaHCO_3$ in 100 ml. of H_2O and sterilize at 121° C. for 15 minutes.

Sterile Sodium Sulfide

Dissolve 0.1 gm. of $Na_2S\cdot 9\ H_2O$ in 100 ml. of water and sterilize at 121° C. for 15 minutes. This must be prepared immediately before use.

Sterile Ethyl Alcohol

Add 2.0 ml. of ethyl alcohol to 100 ml. of water and sterilize by Seitz filtration.

Sterile 5 Per Cent H_3PO_4

Prepare the solution and sterilize at 121° C. for 15 minutes.

Melt the yeast extract agar base and cool to 50° C. Warm the other solutions to 50° and add to the agar. Adjust the pH to between 6.8 and 7.2 with sterile 5 per cent H_3PO_4 with all aseptic precautions observed. Dispense aseptically.

ISOLATION MEDIUM

Prepare a medium similar to the maintenance medium, but omit the yeast extract and agar. Dispense in glass-stoppered bottles and inoculate with mud. Completely fill the bottles so as to exclude air. Incubate under continuous illumination from 25-watt tungsten filament lamps at 25 to 30° C.

When the reddish pigmented growth is apparent examine microscopically for *Rhodomicrobium*. The medium is not specific and permits the development of other photosynthetic organisms. *Rhodomicrobium*, if present, tends to predominate after 7 days of incubation.

To isolate pure cultures, use the maintenance medium dispensed as deeps in soda glass tubes and follow the shake tube procedure outlined for *Chromatium*.

Selection of colonies of *Rhodomicrobium* is facilitated by the deep red color and characteristic convoluted surface of the colonies.

Reproduced by permission from E. Duchow and H. C. Douglas, J. Bacteriol., *58*, 1949, 409, and from R. G. E. Murray and H. C. Douglas, J. Bacteriol., *59*, 1950, 157.

Isolation and Growth of *Rhodopseudomonas*

Medium for Enrichment: Use media and techniques identical to those recommended for *Rhodospirillum*. Ethyl alcohol is the recommended substrate.

Medium for Isolation: Since most species of *Rhodopseudomonas* can be cultured aerobically in the dark or anaerobically with exposure to light, two alternatives are available for final isolation.

Isolate anaerobically in the same manner as species of *Rhodospirillum* or culture aerobically on the following medium.

Difco peptone	10 gm.
Difco yeast extract	5 gm.
NaCl	5 gm.
H_2O	1000 ml.
Agar	15 gm.

Dissolve at 121° C. for 15 minutes, clarify, and adjust the pH to 7.0.

Sterilize at 121° for 15 minutes.

Check red colonies harvested from this medium for their ability to grow anaerobically with exposure to light.

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Isolation and Growth of *Chlorobium*

This organism is a photosynthetic, strictly anaerobic green bacterium containing *Chlorobium* chlorophyll which has its absorption band at approximately 750 mμ (in the invisible infrared region). It oxidizes sulfide with deposition of sulfur outside the cell. It occurs in fresh water and marine muds.

PREPARATION OF ENRICHMENT CULTUR

Liquid Medium for Enrichment

Since the conditions of enrichment highly selective, prepare the follow liquid medium and use without steril tion.

NH_4Cl	1.0
KH_2PO_4	1.0
$MgCl_2$	0.5
$NaHCO_3$	2.0
$Na_2S \cdot 9\ H_2O$	1.0
NaCl	10 to 30 gm. depending on hab
$FeCl_3 \cdot 6\ H_2O$	0.0005
Tap water	1000

Adjust the pH to 7.3 with phosph acid.

NOTE: Prepare this medium just be use. Do not attempt to store it, since very unstable.

Select mud from a locality where th is free exposure to light and active dec position of organic matter with liberat of H_2S. Place a layer 0.5 to 1.0 cm. dee the bottom of 50-ml. wide-mouthed grou glass-stoppered bottles, and fill the bot to capacity with the liquid enrichm medium. Insert the stoppers to exclude and incubate at 25 to 30° C. with bottles placed approximately 4 inches fr 25-watt tungsten filament lamps.

Green bacteria will usually appear i to 7 days. If there is an overgrowth of or purple bacteria different sources of material should be tested.

Even in cultures in which green bact appear, the red and purple organisms subsequently develop. Do not delay is tion unnecessarily.

PURE CULTURE ISOLATION

Chlorobium Agar

NH_4Cl	1.0
KH_2PO_4	1.0
$MgCl_2$	0.5
NaCl	0 to 30 gm. depending on sou of inocu
Agar	10 to 15
Tap water	700

clave to dissolve. If the agar is not
used immediately, sterilize at 121° C.
minutes.
pare the following solutions imme-
before use and sterilize at 121° C.
minutes: (*a*) $NaHCO_3$, 2 gm.: H_2O,
l.; (*b*) $Na_2S \cdot 9\,H_2O$, 1 gm.: H_2O, 100
) $FeCl_3 \cdot 6\,H_2O$, 5 mg.: H_2O, 100 ml.;
) N H_3PO_4.
use melt the agar and cool to 45° C.
ater bath.
rm solutions *a*, *b*, and *c* to 45°, add
cally to the agar, and mix well.
ust the pH to 7.3 with sterile phos-
acid.
tribute the medium aseptically in 10-
nounts in sterile 150- by 16-mm. soda
tubes held in the water bath at 45° C.
ke the isolation by the shake tube
od (see methods of isolation for
atium).
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n, Kgl. Norske Videnskab. Selskabs,
er, 1953; and from H. Larsen, C. S.
m, and C. B. van Niel, J. Gen.
iol., *36*. 1952, 161.

Determination of Thiosulfate, ate, Sulfite, Sulfide, Sulfur, Poly- nate, and Dithionate

erence: J. L. Mangan, N. Z. J. Sci.
, Sect. B, *30*, 1949, 323.
e following pattern of analysis is based
ly on procedures developed by Man-
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methods for elemental sulfur are
ed from the sources indicated.
e initial determination of pH is im-
nt since it gives an indication of the
re of the organism and the end prod-
produced.
e insoluble elemental sulfur in the
ium and the bulk of the cystine are
ved by centrifugation or filtration and
mined separately.
l inorganic sulfur compounds other
S^0 are determined by Mangan's proce-
s about which he comments as follows:

"The method described below uses only one sample of the solution, and upon this can be carried out estimations of dithionate, polythionate (includes tri-, tetra- and pentathionates), thiosulfate, sulfate, sulfite and sulfide. The method depends on the solubilities of the lead salts, and the above compounds can be separated into four groups.

"Group I consists of dithionate and the higher polythionates since their lead salts are freely soluble in water and aqueous alcohol.

"Group II consists of lead sulfate and thiosulfate which, although slightly soluble in water, are quite insoluble in 65 per cent v/v alcohol-water.

"Group III consists of lead sulfite, which is insoluble in water and aqueous alcohol but soluble in sodium hydroxide solution.

"Group IV consists of lead sulfide which is insoluble in sodium hydroxide solution.

"Group II is separated from Groups III and IV by its preferential solubility in ammonium acetate solution, lead sulfate and thiosulfate being very soluble, while lead sulfite is only slightly soluble and lead sulfide insoluble.

"The groups thus separated are analysed for their components. In group I polythionate is determined in the presence of dithionate by its oxidation to sulfate under alkaline conditions. After determination of the sulfate formed, dithionate, which is quite stable to oxidation is hydrolysed by the reaction

$$H_2S_2O_6 + H_2O \xrightarrow{\text{acid}} H_2SO_4 + H_2SO_3$$

and the sulfur dioxide determined by the Monier-Williams method* for sulfite. In group II thiosulfate is determined by titration with iodine, and sulfate then determined by the barium chloride method. Sulfite in group III is estimated by iodine titration. Sulfide in group IV is oxidized to sulfur by excess standard iodine in acid solution, and the excess iodine remaining is determined by thiosulfate titration."

---

* Official Methods of Analysis, Association of Official Agricultural Chemists, 7th ed., 1950, p. 471.

Procedure: (*a*) Determine the pH of the culture. (*b*) Set aside a representative sample for total sulfur determination. (*c*) Centrifuge the remainder at 5000 r.p.m. to remove visible solids. Decant, resuspend the residue in distilled water, and recentrifuge. Repeat the washing and then mix the original supernatant and washings and dilute to a defined volume.

The liquid and solid fractions are treated separately.

Use of the Liquid Fraction: Use one 10-ml. aliquot of the liquid to determine the inorganic sulfur compounds by Mangan's procedures. If the concentration of sulfite and sulfide prove to be negligible a second aliquot may be used for a separate determination of the sulfate. A third aliquot may be used for the determination of dissolved cystine.

Use of the Solid Fraction: The insoluble cystine is extracted from the insoluble residue with dilute HCl and diluted to a defined volume for analysis.

The residue after acid extraction is dried *in vacuo*; sulfur is extracted with carbon tetrachloride and subsequently oxidized to sulfate, precipitated with barium, and weighed.

ANALYSIS OF THE INORGANIC SULFUR COMPOUNDS BY MANGAN'S METHODS

Reagents

Lead Acetate: Dissolve 20 gm. of pure lead acetate in 65 ml. of water with warming and make to a volume of 200 ml. with alcohol.

Ammonium Acetate: Dilute 286 ml. of glacial acetic acid with an equal volume of water and add 0.88 ammonia until the pH is approximately 6.0. Bromthymol blue can be used as an indicator and ammonia added until a green color is obtained. If excess ammonia is added the excess may be boiled off. Make the solution to 1 L. It is approximately 5 M.

Benzidine Reagent: Use 1.6 gm. of benzidine to 20 ml. of N HCl and add 45 ml. of water. Warm the solution to dissolve the benzidine and make the volume to 200 with alcohol.

Method

Pipette 10 ml. of the approximat neutral solution into a 50-ml. centrifu tube and add 20 ml. of 95 per cent ethan Add 0.5 ml. of 10 per cent NaCl as a car and mix. Add excess lead acetate reage usually 8 ml. is enough. Centrifuge at 2 r.p.m. for 15 minutes; check compl precipitation with a drop of lead aceta and decant the supernatant into a 250- beaker. Resuspend the precipitate u formly in 65 per cent ethanol and recent fuge. Combined supernatant and washin constitute Group I containing di-, t tetra-, and pentathionates.

Suspend the insoluble lead salts in 8 of ammonium acetate reagent. Dilute mixture to 40 ml. with distilled water c taining 5 per cent sucrose or glycerol prevent oxidation of sulfite. Add 2 to drops of isobutanol to prevent surfa effects and spin down the solid material a centrifuge. Commercial wetting age should not be used as they often interf with the starch iodide end point and so also contain sulfate. Decant the supern tant into a 250-ml. beaker and wash solids with glycerol water. The ammoni acetate extract and washings make Gro II containing sulfate and thiosulfate.

Suspend the remaining solid material 1.0 ml. of 10 per cent NaOH and dilute 40 ml. with glycerol water. In the abse of sulfide this is Group III containi sulfite. When sulfide is present centrif the tubes, decant the supernatant, wash the residue with glycerol water a add to the supernatant to give Gr III. The insoluble residue is Group IV.

Method for Group I

To remove excess lead, add bromthy blue indicator followed by a suspension NaHCO₃ until the indicator changes blue-green. Filter the lead carbonate p cipitate with suction and wash it with

ent ethanol, collecting filtrate and
ngs in a 250-ml. beaker. Add 0.5 gm.
e sodium peroxide, and oxidize the
polythionates to sulfate by heating
C. on a water bath for 15 minutes.
nate is not affected by this treatment.
compose the remaining peroxide add
s of 1 per cent cobaltous acetate and
until all signs of peroxide decomposi-
ease. Place in an ice bath and cool to
add concentrated HCl dropwise until
acid to bromphenol blue. The pH
d be near 2.8 which gives the minimal
ility of benzidine sulfate. Slowly
luce benzidine reagent in 1-ml. ali-
at 2-minute intervals with stirring.
milliliters is usually enough for quanti-
f polythionate sulfur in the range of
to 1.75 mg. in about 70 ml. Leave in
ater for 15 minutes and filter through
pared Gooch crucible. After washing
aqueous alcohol suspend the benzidine
te and asbestos in 100 ml. of distilled
r, and titrate near the boiling point
0.02 N NaOH and the use of phenol
ndicator. Near the end point boil for a
minutes before completing the titration.

f 0.02 N NaOH × 0.32

= mg. of polythionate-S

ionate decomposes under strong acid
itions as follows:

$$O_6 + H_2O \xrightarrow{HCl} H_2SO_4 + H_2SO_3$$

e the filtrate and washings from the
ious test alkaline with NaOH and
l off the alcohol. Wash the aqueous
ion into a 500-ml. 3-necked flask
pped with a dropping funnel, an inlet
CO_2, and a reflux condenser fitted at
top with a down tube dipping into 3
cent hydrogen peroxide. Bring the total
ume to 200 ml. with water. Pass a
am of CO_2 to remove all air and bring
liquid to the boil. Introduce 35 ml. of
(specific gravity 1.17) through the
pping funnel without admitting air.
ux for 1 hour. Then cut off water from the condenser. Just before steam enters the peroxide disconnect the apparatus from the heat source. SO_2 arising from dithionate decomposition is driven over into the peroxide where it is oxidized to sulfuric acid. A blank determination is required. Determine the sulfuric acid by titration with 0.02 N NaOH with the use of bromophenol blue as an indicator *or* precipitate as barium sulfate and weigh.

ml. 0.02 N NaOH × .321 × 2

= mg. dithionate-S

137.35 × 2 × gm. of $BaSO_4$

= mg. of dithionate-S

Method for Group II

Thiosulfate: The ammonium acetate extract has a pH of 6.0. Lead salts do not affect the titration of thiosulfate, but the pH is of importance.

Adjust the pH below 5.0 by adding 10 ml. of 20 per cent v/v HCl. Add starch indicator and titrate with 0.02 N iodine.

1.282 × ml. of 0.02 N I_2

= mg. of thiosulfate-S

Sulfate: This can be determined with sufficient accuracy in the presence of ammonium and lead salts. To the solution after titration of the thiosulfate, add 5 ml. of concentrated HCl followed by an excess of 2.5 per cent $BaCl_2$ added dropwise in the cold. Set aside for 1 hour. Then filter onto a weighed Gooch crucible, wash thoroughly with hot water, ignite at 600° C., and weigh.

If lead chloride should precipitate when the concentrated hydrochloric acid is added, dilute and warm to dissolve it. The precipitation of the barium sulfate must be carried out at room temperature.

Sulfate may also be determined directly on aliquots of medium free of sulfide and sulfite. Dilute 20 ml. to 100 ml. with water. Acidify with 0.5 ml. of concentrated HCl and treat with $BaCl_2$ in the cold to precipitate sulfate. Allow the sample to stand for 24 hours and filter through 9-cm. No. 40 Whatman paper. Wash with hot water, dry, and incinerate. When the ash is white, add 1 drop of concentrated H_2SO_4 and incinerate, driving off excess acid.

Method for Group III

Sulfite is determined on the alkaline extract. Dilute to 75 ml. with water containing sucrose or glycerol and add dilute HCl with stirring until acid to bromphenol blue. Add starch indicator and titrate with 0.02 N iodine.

$$0.321 \times \text{ml. of } I_2 = \text{mg. of sulfite-S}$$

Method for Group IV

Suspend the black precipitate of lead sulfide in a small amount of water and wash into a 250-ml. Erlenmeyer flask with a stopper. Add 10 to 15 ml. of 0.02 N iodine followed by 10 ml. of 20 per cent v/v HCl. Close the flask and allow to stand for several minutes. Dilute to 100 ml. and back titrate with 0.02 N thiosulfate. A blank determination should be made.

$$0.321 \times \text{ml. of } I_2 = \text{mg. of sulfide-S}$$

Elemental Sulfur: Vacuum dry the residue remaining after acid extraction of the insoluble material in the culture to remove cystine. Place the dried residue in a thimble in the Soxhlet apparatus and extract at least 12 times with carbon tetrachloride. Separate the extract and evaporate to dryness. Heat it in an oven at 60° to 70° C. for 20 minutes, then cool to room temperature.

Working in a fume cupboard add 10 ml. of a saturated solution of bromine in carbon tetrachloride, cover, and allow to stand for 30 minutes with intermittent agitation. Add 15 ml. of concentrated nitric acid and allow to stand for an additional 30 minutes with intermittent agitation. Evaporate on a hot plate to approximately 5 ml. Add 20 ml. of concentrated HCl and evaporate to approximately 5 ml.
Add 50 ml. of distilled water, filter, a
wash with 2 per cent HCl. Add 2 drops
bromothymol blue indicator (0.1 gm.
indicator dissolved in 16.0 ml. of 0.01
NaOH and diluted to 250 ml.) and th
ammonia until the solution turns blue. A
HCl 1 drop at a time until the solution
distinctly acid and then add 5 drops
excess. Dilute to 150 ml. and heat to bo
ing; add 10 per cent $BaCl_2 \cdot 2\,H_2O$ soluti
dropwise until no more precipitate for
and then add 50 per cent excess. Cover t
beaker and digest on a steam bath for
least 1 hour. Cool and filter through
asbestos filter in a Gooch crucible which
been ignited at 500° C. and weighed. Wa
thoroughly with hot water. Ignite in
muffle furnace at 500° C. for at least
minutes. Cool in a desiccator and wei
The difference in weight is S as $BaSO_4$

Reference: Methods of Analysis, A.O.A.
7th ed., 1950. Reproduced with a modific
tion by permission from the Association
Official Agricultural Chemists. Note: t
substitution of CCl_4 for CS_2 is made by t
author and is *not* sanctioned by the Assoc
tion of Official Agricultural Chemists.

Total Sulfur: Place the sample in a 2
ml. Kjeldahl flask fitted with a W goo
neck condenser and glass beads. Into t
goose neck, place 0.5 ml. of 10 per ce
iodic anhydride and add enough water
fill the two bottom elbows. Into the fl
place 0.5 ml. of 10 per cent iodic anhydr
followed by 2 to 3 ml. of a mixture o
parts of 66 per cent perchloric acid an
part of fuming nitric acid. Join the fl
and condenser, and bring the flask t
gentle boil. Heat until the digestion liq
becomes colorless or only slightly yell
Cool a little and then empty the conden
contents into the flask and flush with
little water. Heat again slowly until onl
few milliliters remain. Add 25 ml. of wa
followed by 10 per cent hydrazine hydr
added dropwise to reduce the iodic acid
iodide. Enough is added to dispel the yell
color which first appears. Neutralize
phenolphthalein, acidify slightly with H

nd treat with a slight excess of 5 per arium chloride to precipitate sulfate.

35 × gm. of $BaSO_4$ = mg. of sulfur

tine: In a 25-ml. volumetric flask a 5.0-ml. sample containing 0.2 to g. of *l*-cystine in dilute HCl. Add 3.0 5 per cent NaOH from a burette and to stand for 15 minutes at room rature with occasional swirling. Then ml. of sodium β-naphthoquinone-4- ate (containing 3 mg. per ml. of the hoquinone reagent purified by re- llization from 50 per cent alcohol) ed within 10 seconds by 8.0 ml. of r cent Na_2SO_3 in 0.5 N NaOH. Allow to develop for 45 minutes. Add 3.0 f 5 N NaOH followed by 2.0 ml. of er cent sodium hydrosulfite in 0.5 N I, and adjust the volume to 25 ml. water. Mix and read within 2 minutes Klett-Summerson colorimeter with a ten 58 filter. Compare with standard ols.

produced by permission from M. X. an, Public Health Repts. U. S., *44*, 1421; and from F. A. Csonka, H. enstein, and C. A. Denton, J. Biol. n., *156*, 1944, 571.

ction and Crystallization of Ele- tal Sulfur from Organisms which sit Sulfur Internally

merse a clean slide in clarified agar; and remove the agar from one side. a small drop of a dense suspension of ulture on the agar and cover with a slip. Place a drop of water against one f the coverslip and a drop of pyridine st the other. Set the slide up under the r high power of the microscope.

ace a piece of filter paper on the slide st the water drop, so that the water awn away from the coverslip. The ine is thus drawn across the slide r the coverslip.

lfur granules will dissolve in the pyridine but should recrystallize extracellularly as rhombic or monoclinic crystals. The rapid recrystallization is thought to be due to the absorption of the water from the pyridine-water mixture by the agar so that the pyridine is left supersaturated with sulfur.

Reproduced by permission from V. B. D. Skerman, G. Dementjeva, and B. J. Carey, J. Bacteriol., *73*, 1957, 504.

The Production of Sulfide (H_2S)

Microbial production of sulfide results from the reduction of sulfate or the decomposition of organic sulfur compounds such as cystine, methionine, and glutathione. Since the nutritional requirements of organisms which are potentially capable of producing hydrogen sulfide are very broad, any one medium will not suit all types. The recommendations are for this reason directed towards the test for the sulfide rather than media used for the growth of the organism. Nevertheless, for taxonomic purposes, comparative reactions on specific media are desired and suggestions have been made for consideration.

H_2S *per se* does not exist in a medium at pH 7.0. The sulfur is present predominantly as the ^-SH ion. The solubility of this will determine the extent to which H_2S will be liberated from the medium. This is important in the application of the lead acetate strip technique.

H_2S production does not occur when an adequate supply of oxygen is available for cell metabolism. Therefore conditions of incubation which permit a high degree of aeration should not be used.

Growth "aerobically" on the surface of an agar plate does not necessarily constitute aerobic conditions within the colony. On the contrary, the availability of oxygen to cells within a colony more than one cell deep is very poor. This permits the production of H_2S and the formation of black colonies on iron-containing media, *e.g.*, glucose-blood-cystine agar for *Pasteurella tularensis*.

MEDIA

1. *Mineral Salts Media:* The essential feature of any mineral salts medium is that it contains at least one of the salts in the form of a soluble sulfate in a concentration not less than 500 mg. per L. If an iron salt is present the production of sulfide will result in the production of black iron sulfide.

If the concentration of iron is too small it may be necessary to use the lead acetate paper method or to test the culture for sulfide by the methylene blue procedure.

2. *Organic Media:* Any organic liquid medium containing sufficient cystine may be used, but for comparative purposes the following media are recommended: (*a*) peptone water plus .01 per cent cystine and 0.05 per cent sodium sulfate; (*b*) ferric ammonium citrate agar; and (*c*) ferrous chloride gelatin.

In the two iron-containing media production of sulfide is indicated by the production of black iron sulfide. Production of sulfide in the peptone medium may be detected by the lead acetate paper strip method or the methylene blue method.

THE LEAD-ACETATE PAPER METHOD

Cut filter paper into 50- by 10-mm. strips and immerse them in a 5 per cent solution of lead acetate. Dry in air. Sterilize in a suitable container at 121° C. for 15 minutes.

Following inoculation of the liquid medium, insert a strip of the sterilized paper between the plug and the glass with the lower end above the liquid level. Incubate.

If H_2S is liberated during the growth of the organism, the lower portion of the paper will turn black. If, after incubation there has been no color change, remove the plug and add 0.5 ml. of 2 N HCl; replace the paper and plug without delay. The addition of the acid will liberate any dissolved sulfide which will react with the lead to yield the black lead sulfide.

If heat is applied to assist the reaction, use a control tube of uninoculated medi
to eliminate any possibility that the re
tion is caused by decomposition of
medium.

THE METHYLENE BLUE METHOD

The test is based on the reaction in a
solution between *p*-amino-dimethyl ani
and sulfide in the presence of ferric chlor
to form methylene blue. The test is
tremely sensitive and 0.01 p.p.m. of sul
may be detected. The color develope
proportional to the sulfide concentratio

Test

To the 5 ml. of the culture add 0.5
of amine-sulfuric acid solution and 0.1
of ferric chloride solution. Spin the t
to mix.

Results

A blue color is positive for H_2S, pin
negative for H_2S.

Reagents

Stock Amine-HCl Solution: The c
mercial *p*-amino-dimethyl aniline may
received as a mixture of crystal and liq
Drain the crystals and press dry betw
filter papers. If the original materia
entirely liquid, cool until it has parti
solidified. If the dry crystals are dark, r
them in a closed vessel and again parti
solidify by slow cooling. Seeding may
necessary to prevent excessive supercool
Again dry the crystals. Add a kn
amount of the amine gradually to con
trated HCl in a beaker surrounded
ice. The final solution should contai
gm. of amine in 100 ml. of solution.

Amine-Sulfuric Acid Solution: Add
ml. of concentrated H_2SO_4 to 480 ml
water and cool; add 20 ml. of amine-
solution.

Ferric Chloride Solution: Dissolve 45
of $FeCl_3$ (*or* $FeCl_3 \cdot 6\,H_2O$, 75 gm.) in enc
water to make 100 ml. of solution.

Reference: R. Pomeroy, Sewage W
J., *8*, 1936, 572. Reproduced by permi

Federation of Sewage and Industrial s Association.

ne-Cystine-Sulfate Medium for e Production

ne	10 gm.
e	0.1 gm.
$_4$	0.5 gm.
ed water	1000 ml.

olve the ingredients. Adjust the pH and filter if necessary. Dispense in amounts in 150- by 13-mm. tubes and e at 121° C. for 15 minutes.

rence: Author's recommendation.

us Chloride Gelatin for Sulfide ction and Gelatin Liquefaction

's meat extract	7.5 gm.
ne (Parke-Davis)	25.0 gm.
	5.0 gm.
ater	1000 ml.
n	120 gm.
$eCl_2 \cdot 4\ H_2O$	5 ml.

solve all ingredients except the ferric de. Adjust the pH to 7.6 and filter if ary. Sterilize at 110° C. for 25 es.

nediately before use melt the medium dd the required amount of sterile s chloride solution. Dispense asepti- in 150 by 13-mm. tubes and cool tly. Seal the tubes with sterile stop-

culate by stabbing and incubate for s at about 20° C.

roduced by permission from F. Kauff- The Enterobacteriaceae, 2nd ed., sgaard, Copenhagen, 1954.

Ammonium Citrate Agar (for de Production)

ck colonies on ferric ammonium ci- agar indicate the production of hy- n sulfide by the organism.

ne	10.0 gm.
extract	2.0 gm.
m chloride	5.0 gm.
ammonium citrate	0.3 gm.
Agar	14.0 gm.
Tap water	1000 ml.

Dissolve by heating to 121° C. for 15 minutes. Adjust the pH to 7.0 and filter if necessary. Dispense in 5-ml. quantities in 150 by 13-mm. tubes. Sterilize at 120° C. for 15 minutes.

Inoculate the tubes by stabbing the medium with a wire. H_2S production is indicated by the production of black iron sulfide.

Reference: The origin of this medium is not known.

Peptone Water

Peptone	10 gm.
NaCl	5 gm.
Tap water	1000 ml.

Dissolve the ingredients and adjust the pH to 7.2. Filter if necessary. Dispense in 150- by 13-mm. tubes and sterilize at 121° C. for 15 minutes.

0.1 Per Cent Peptone Agar

Peptone	1.0 gm.
Agar	14 gm.
Tap water	1000 ml.

Dissolve by heating to 121° C. for 15 minutes. Adjust the pH to 7.0 and filter. Sterilize at 121° C. for 15 minutes.

Peptone Yeast-Extract Acetate Agar

Bacto yeast-extract	5.0 gm.
Bacto-peptone	5.0 gm.
Sodium acetate	0.1 gm.
Agar	15 to 20 gm.
Distilled water	1000 ml.

Dissolve by heating to 121° C. for 15 minutes. Adjust the pH to between 7.4 and 7.6 and filter if necessary. Sterilize at 121° C. for 15 minutes.

Reproduced by permission from E. G. Pringsheim and C. F. Robinow, J. Gen. Microbiol., *1*, 1947, 267.

Papain Digest Broth and Agar

The Concentrate: Mix 500 gm. of defatted and minced ox heart with 1000 ml. of distilled water and 1 gm. of papain. Digest in a water bath at 60° C. for 15 to 24 hours. Add 5 gm. of NaCl and steam for 90 minutes. Filter while very hot through paper pulp or clarifying pad (Ekwip DO).

Add 35 ml. of N NaOH and autoclave at 121° C. for 20 minutes. Filter while hot. Cool and adjust the pH to between 7.4 and 7.6 with N HCl. Distribute in 1 L. screw cap bottles for storage. Sterilize at 121° C. for 15 minutes.

Dilution of the digest for use is based on the amino-N content. A dilution containing 1 mg. of NH_2-nitrogen per 10 ml. is suitable for most purposes in place of broth and peptone waters. It is excellent for indole tests.

Estimation of Amino-Nitrogen: Place a 10-ml. sample in a 150-ml. conical flask. Add 30 to 40 ml. of distilled water and 0.5 ml. of phenolphthalein indicator. Titrate with 0.1 N NaOH from a burette until a pink end point is reached. Take the reading (R_1) and then add 10 ml. of neutral formalin to the mixture; titrate again to the same end point (R_2). If the NaOH is 0.1 N.

$$1.4\ (R_2 - R_1) = \text{mg. of amino-N in 10 ml.}$$

For use: Dilute the concentrate to contain 4 mg. of amino-N per 10 ml. Adjust the pH to 7.0. Filter if necessary and distribute. This is papain digest broth.

To prepare papain digest agar, add 2.0 per cent agar to the broth before adjusting the pH. Autoclave at 115° C. for 20 minutes to dissolve the agar. Adjust the pH to 7.0, filter if necessary, and sterilize at 115° for 20 minutes.

Meat Infusion Agar

Free a bullock's heart of fat and mince it. To every 500 gm. of mince add 1000 ml. of water, 10 gm. of peptone, and 5 gm. of NaCl. Steam for 60 to 90 minutes, stirring occasionally to break up the clump of meat which forms. Filter through cotton wool. Add N NaOH till the solution is just pink to phenolphthalein (usually 25 ml. for heart meat). Heat to 121° C. for 15 minutes and filter while very hot through a pleated filter paper. Readjust the pH to betwee 7.4 and 7.6. Add 14 to 20 gm. of agar pe L., heat to dissolve, filter if necessary, an sterilize at 121° for 20 minutes.

Reproduced by permission from H. D Wright, J. Pathol. Bacteriol., *37*, 1933, 257

Media for the Detection of Lipolytic Organisms

BASAL MEDIUM

Peptone	15 gm.
Yeast extract	5 gm.
Sodium chloride	5 gm.
Agar	15 gm.
Water	1000 ml.

Dissolve the ingredients at 121° C. for 20 minutes. Adjust the pH to 7.5 and filter. Add the required amount of fat, mix well, and sterilize at 121° for 20 minutes.

PREPARATION OF NILE BLUE SULFATE

Prepare a saturated aqueous solution of nile blue sulfate. Precipitate the oxazine base from it by the dropwise addition of N NaOH until precipitation is complete. Filter and wash the precipitate with distilled water with the pH adjusted to 7.5. Dry and store for use.

Fats vary in the intensity with which they stain. Tripropionin, tributyrin, tricaproin, tricapyrlin, triolein, beef tallow, butter fat, coconut oil, corn oil, cottonseed oil, lard, linseed oil, and olive oil all stain bright red.

Tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin decrease in the intensity of staining with the increase in molecular weight.

PREPARATION OF THE DYED FAT

Prepare a saturated solution of the nil blue sulfate oxazine base and mix 1 m with 10 ml. of the fat. If necessary wor in a heated water bath to liquefy the fa and maintain it in the liquid state through-out the washing process.

With tributyrin and similar triglycerides which are liquid at room temperature, ad double the quantity of ether to the fat-d in a separatory funnel; separate the r-soluble fat layer from the water d wash several times with water. separate the ether-fat layer and e off the ether. (Keep away from Separate the fat from the residual nd sterilize at 121° C. Store in a tor. For use add 1 ml. of the dyed ml. of the melted basal medium, , and pour.

fats which solidify at room temwash the dyed fat with several of heated water and then disperse n a melted 0.5 per cent neutral agar in the proportion of 10 ml. of fat l. of agar.

ize the mixture at 121° C. for 20 , cool until solidified, and then igorously until the fat is emulsified. se melt the fat emulsion and the medium, and add 1 ml. of the fat n to 20 ml. of the basal medium. ll and allow to set.

: With Nile blue sulfate the fat ed and fatty acids stain blue. Hyof the fat will result in a blue halo the colonies. In some cases the in cloudiness of the medium may be amatic than the color change.

r methods can be employed with es of neutral red, methylene blue, lachite green prepared as for Nile fate. In the respective cases the fats are orange-yellow, red, and een, and the fatty acids red, blue, e-green. One of these may prove if Nile blue is reduced.

duced by permission from M. A. and B. W. Hammer, J. Bacteriol., 4, 473; and from G. Knaysi, J. ol., *42*, 1941, 587.

ction of Acid or Acid and Gas Carbohydrates, Sugar, Alcohols, kaloids

: Production of acid may be the rean oxidative process (*e.g.*, glucose onic acid) or a fermentative one. eat majority of descriptions of biochemical properties in which acid production is mentioned refer to the latter type of reaction. The fermentative reactions occur simply because the medium is dispensed in such a manner that solution of O_2 from air by diffusion rarely meets the demand of the growing cell population. Although tubes are incubated aerobically, processes occurring in the tubes are essentially anaerobic.

The necessity to discriminate between the oxidative and fermentative production of acid is becoming more apparent. The method suggested by Hugh and Leifson (p. 172) has considerable practical merit.

In any method used for the detection of acid production the observation is usually restricted to a visible change in the color of an acid-base indicator. If the test is performed in an inorganic basal medium, the degree of color change will be dependent on the buffer capacity of the medium. For this reason the concentration of buffering salts such as phosphates should be reduced to a minimum consistent with metabolic requirements.

In media which contain amino-nitrogen the liberation of ammonia may, in some cases, be sufficient to neutralize all the acid produced and yield a false negative reaction. If a negative reaction is thought to be due to this, retesting in a mineral salts medium (if growth occurs) or in a medium with a smaller amount of organic nitrogen as suggested by Hugh and Leifson may yield a positive result.

In all the media recommended for acid production in these notes the substrates are sterilized in concentrated (usually 10 per cent) solutions and added aseptically after the sterilization of the medium. Sterilization by filtration is recommended but, provided the solutions are prepared in distilled water and do not turn yellow, heat sterilization at 110° C. for 25 minutes is satisfactory for other than di- and polysaccharides which may undergo a degree of hydrolysis. Heat sterilization of the substrate in the peptone base is widely practiced and, where positive results are obtained, is usually satisfactory. However, with some organisms negative reactions in such media are meaningless, because the solution is rendered toxic by the heat treatment.

METHODS

Mineral Salts Base

The basal mineral salts medium described by Pope and Skerman (p. 143) is recommended for this purpose. Add 2.0 ml. of a 1.6 per cent alcoholic solution of bromocresol purple per L. before final adjustment of the pH. Distribute in 5-ml. amounts in 150 by 13-mm. tubes, insert a Durham tube, and sterilize by heating to 121° C. for 20 minutes. Add the filtered substrates aseptically to a final concentration of 1.0 per cent.

Acid production is indicated by a change in the color of the indicator from purple to yellow. Gas, if produced, accumulates in the Durham tube.

Peptone Base

Prepare as above with the 1 per cent peptone water used in place of the mineral salts base.

Hiss's Serum Water Base:

This base is employed for organisms which will not grow or which grow poorly in peptone water.

Peptone	5.0 gm.
K_2HPO_4	1.0 gm.
Water	1000 ml.

Dissolve by steaming for 15 minutes. Adjust the pH to 7.4. Add 8 ml. of Andrade's indicator (*vide infra*). Sterilize at 115° C for 10 minutes. Cool to 50° and add 100 ml. of sterile ox serum. Add the substrates aseptically to a final concentration of 1 per cent and dispense aseptically in 5-ml. amounts in sterile 150- by 13-mm. tubes.

Andrade's Indicator

Acid fuchsin	0.5 gm.
Distilled water	100.0 ml.
N NaOH (4 gm./100 ml.)	1000.0 ml.

The Durham tube is omitted from th basal medium, because acid productio which changes the color from colorless t pink may be accompanied by a coagulatio of the serum which renders the gas tub inoperative.

Other Bases

Organisms which will not grow in any o the above bases must be tested in a base i which they will grow. In reporting such results a simultaneous report should b made of other bases tested and foun unsuitable.

The Eldredge Tube for Detection o Carbon Dioxide Evolution

The Eldredge tube is illustrated in figure 3.

Place 20 ml. of the medium in one of th horizontal arms, plug both vertical arm with cotton wool, and sterilize. Inoculat the medium and then pipette 20 ml. o freshly prepared 0.1 N barium hydroxid into the second horizontal arm. Cut off th tops of the cotton wool plugs, push then down slightly into the tubes and seal th vertical arms with rubber stoppers. Incubate for 14 days.

Copious evolution of CO_2 from the culture will result in a precipitation of barium carbonate in the horizontal arm. Titrate the residual barium carbonate with 0.1 N hydrochloric acid, with the use of phenolphthalein as an indicator. This result wil give only, by difference, the evolved carbo dioxide. The CO_2 dissolved in the mediu should be liberated by acidification prior t titration of the barium hydroxide, if th total CO_2 output is required.

Reproduced by permission from Manu of Methods for the Pure Culture Study o Bacteria, Biotech Publications.

Differentiation of Oxidative and Fermentation Production of Acid fro Carbohydrates

MEDIUM

Peptone	2.0 gm
NaCl	5.0 gr

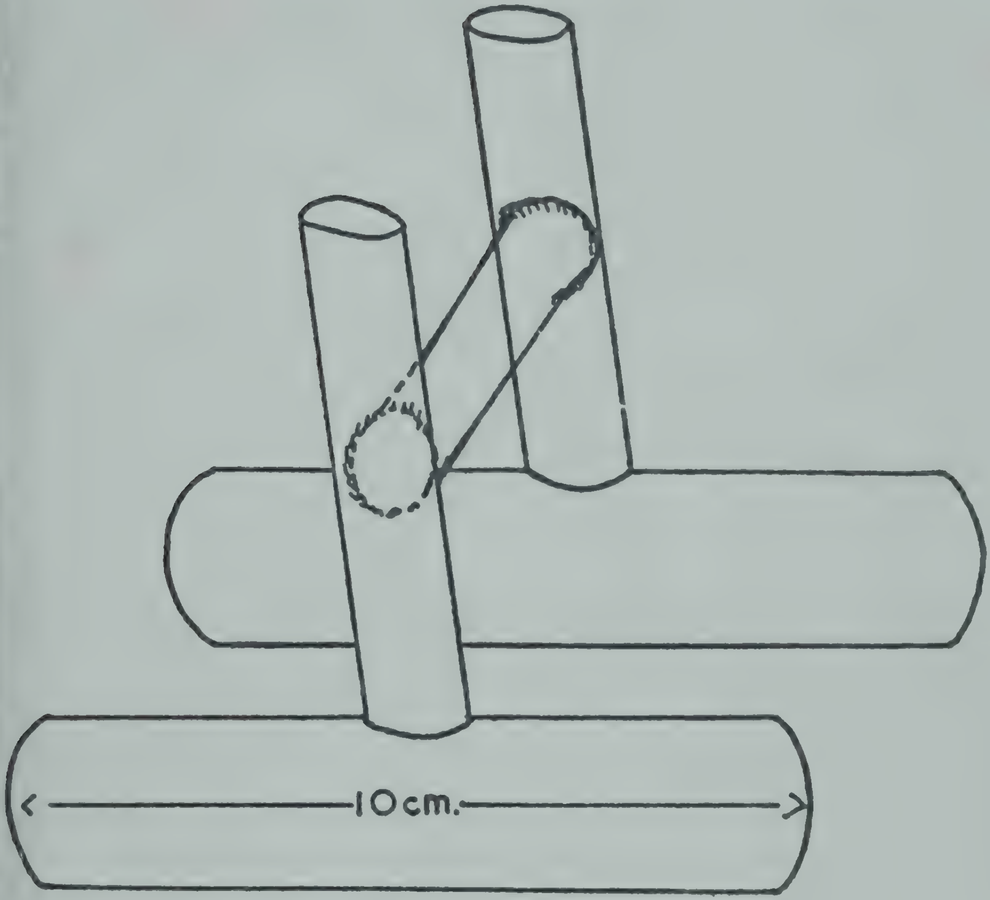


FIG. 3

O_4	0.3 gm.
	3.0 gm.
hymol blue (1% aque- solution)	3.0 ml.
ed water	1000 ml.

olve the ingredients and adjust the necessary, to 7.1. Sterilize at 121° C. minutes.

pare 10 per cent aqueous solutions of rbohydrates and sterilize by Seitz on. Add 10 ml. of the sterile carbo- es aseptically to every 100 ml. of the , melted medium and dispense in 5- nounts in sterile 150- by 13-mm.

culate two tubes of each carbohydrate ach organism by stabbing with inocu- rom a fresh slope culture. Cover the e of one tube with sterile paraffin latum).

NG THE TESTS

mentative organisms produce acid hout both tubes. Oxidative organisms produce acid in the open tube only. In the latter, acid appears first at the surface and then progressively towards the base.

Slow oxidative reactions are sometimes preceded by a slight alkaline reaction.

Author's Note: 1. Dr. P. H. A. Sneath (personal communication) has suggested the use of long narrow tubes and the elimination of the paraffin (petrolatum) seal to avoid the unpleasantness associated with washing the sealed tubes.

2. Although this test sharply differentiates between two groups of organisms, the suggestion that the acid produced by the oxidative organisms is the result of an "oxidative" action of the sugar is open to question. Some organisms which give acid only in the open tubes have been shown to produce no acid at all if grown under strictly oxybiontic conditions in shallow liquid cultures. It is possible that the so-called oxidative organisms may, in some

instances at least, actually ferment the carbohydrate but are unable to produce sufficient *growth* in the absence of oxygen. Once this initial growth is established and oxygen becomes limiting in the dense surface growth, acid *may* be produced *fermentatively*. This question needs further clarification but does not detract from the usefulness of the test.

Reproduced by permission from R. Hugh and E. Leifson, J. Bacteriol., *66*, 1953, 24.

Litmus Milk

For the preparation of this medium use (*a*) fresh machine-separated milk, *or* (*b*) fresh milk, steamed for 20 minutes and allowed to stand for 18 hours for the fat to rise, after which the fat-free milk is siphoned off aseptically, *or* (*d*) good quality *spray-dried* powdered skim milk reconstituted by dissolving 100 gm. per L. of distilled water.

Add to the milk an alcoholic solution of litmus (*vide infra*) sufficient to give a distinct color (usually 40 ml. per L.). Adjust the pH to 7.0 by the addition of 1 N NaOH. Do not rely on visual adjustment. A glass electrode should be used. Steam for 15 minutes on 3 successive days with incubation at 37° C. between steamings.

Alcoholic Litmus Solution: Grind 50 gm. of litmus in a mortar with 150 ml. of 40 per cent alcohol. Transfer to a flask, and boil gently on a steam bath for 1 minute. Decant the fluid and add another 150 ml. of 40 per cent alcohol to the residue; boil again for 1 minute. Decant and mix the extracts. Allow to settle overnight and make up to 300 ml. with 40 per cent alcohol.

Add N HCl drop by drop to adjust the pH to 7.0.

The Identification of Butyric, Propionic, and Acetic Acids in Cultures

Acidify the culture with phosphoric acid to about pH 2. Distill, with the addition of silicone antifoam if necessary.

Estimate the amount of volatile acids in the distillate by titrating an aliquot w standard alkali and phenolphthalein i cator. Extract the bulk of the distil with ether; evaporate the ether and conv the acids to their ammonium salts by addition of the calculated amount o NH_4OH. Prepare solutions of ammoni butyrate, propionate, and acetate neutralizing these acids with N NH_4OH

The unknown acids are separated identified by paper chromatography of t ammonium salts and comparison with ammonium salts of known acids.

Place small spots (5 to 10 µl.) of liquids about 2 cm. from the ends of st of Whatman No. 3 filter paper (17 by cm.), dry briefly in air, fix the other end slits in cork stoppers, and suspend the pers in test tubes of suitable size, with end dipping into the developing solvent.

Prepare the solvent by shaking toget the following reagents, and use the uppe the two layers which separate.

Water	4 parts by
NH_4OH (concentrated)	1 part by
Ethanol (95%)	1 part by
Redistilled *n*-butanol	4 parts by

Allow the solvent to ascend the pa strips for about 4 hours. Remove the st from the tubes, allow to drain for 30 to minutes on filter paper, and spray th with an indicator solution (200 mg. methyl red and 200 mg. of bromothy blue in 500 ml. of a 1:5 solution of form in alcohol; final pH, 5.2). Finally dip strips into the vapor from 3 per c NH_4OH. Mark the orange-red spots mediately.

It is important to remember that ammonium salts of the volatile acids themselves volatile. Hence do not use h when applying spots to paper, do not le the strips for more than 1 or 2 hours tween the time of applying the spots developing the chromatogram, and do dry the chromatogram completely bef spraying with the indicator.

Reproduced by permission from R. E. Duncan and J. W. Porteous, Analyst, 641; and from A. J. G. Barnett and B. Duncan, J. Sci. Food Agr., *5*, 1954,

action and Determination of Opti- Rotation of Lactic Acid

ie culture should contain at least 2 gm. actic acid. Clarify by adding one- tieth of its volume of 25 per cent zinc te. Neutralize to a pH between 7.6 and ith 20 per cent sodium hydroxide, and rifuge off the resulting zinc hydroxide pitate.

idify the clarified solution to pH 2 with ric acid and extract three times with r (total volume of ether about 1 L.). If eciable amounts of volatile acids or inic acid are present, they should be oved beforehand, but this is not neces- with homofermentative bacteria.

ld water (10 ml.) to the ether extract, distill off the ether. Make the remain- solution up to 50 ml., and titrate 1 ml. etermine the total quantity of extracted . Then boil the solution for 10 minutes a calculated excess of zinc carbonate; r off the excess carbonate and wash. porate the filtrate on a water bath until tallization begins. Add alcohol to a entration of 50 per cent and allow the ure to stand overnight to crystallize. r off the product; wash with 95 per alcohol and then with ether, air-dry, place in a desiccator over calcium ride.

test the optical form of the zinc lac- , the water of crystallization and the ific rotation are determined.

Weigh a 2-gm. sample of the salt ac- tely, dry to constant weight at 110° C., calculate the amount of water of crys- ization. Optically active salt contains 2 ecules (12.89 per cent) of water; the tive salt contains 3 molecules (18.17 cent).

Weigh 1 gm. of the anhydrous salt rately, dissolve in distilled water, and te to 25 ml. at 20° C. Determine the tion of polarized light in a polarimeter, and calculate the specific rotation from the observed rotation by the formula

$$\alpha = \frac{100 \cdot a}{b \cdot c}$$

where α is the specific rotation, a is the observed rotation, b is the length of the polarimeter tube in decimeters, and c is the concentration in gm. per 100 ml. of solution.

The rotation of the active zinc salt is *opposite* to that of the free acid and varies slightly with concentration; a 4 per cent solution (w/v) has a specific rotation of ±8.1 to 8.6.

Reproduced by permission from C. S. Pederson, W. H. Peterson, and E. B. Fred, J. Biol. Chem., *68*, 1926, 151.

See also Biochem. J., *26*, 1932, 846; and Ind. Eng. Chem., *27*, 1935, 1492.

The Demonstration of Alcohols in Cultures

Place 20 ml. of the culture in a distilling flask and collect 10 ml. of distillate. Pipette 1 ml. of this into a 250-ml. flask, and add 10 ml. of oxidizing mixture (0.2 gm. of potassium dichromate in 100 ml. of 19 N sulfuric acid). Stopper the flask and mix the contents thoroughly. Allow to stand for 30 minutes; add 100 ml. of water and 0.5 gm. of KI. After the latter is dissolved, titrate the liberated iodine with N sodium thiosulfate, adding 0.5 ml. of starch indicator *just before* the end point. If no iodine is liberated, repeat with use of less of the distillate.

Do a blank determination by substituting 1 ml. of water for the distillate. The difference in titration between the test and the blank is a measure of the amount of dichromate reduced by the alcohol in the distillate: (1 mmole of ethanol = 4 m.eq. of dichromate).

The method is not specific for ethanol, since all volatile alcohols react.

Analysis of Gases for Pure Culture Studies

Gases which are commonly encountered either as byproducts of fermentations or as substrates for oxidation are CO, CO_2, CH_4, H_2, H_2S, N_2, N_2O, and O_2.

The most accurate method of analysis is possibly by means of the mass spectrograph, but this is outside the scope of most laboratories.

The technique of gas chromatography is relatively inexpensive and may prove to be the ideal tool in microbiological work, particularly for the qualitative identification of gases before quantitative analysis. The technique is at present under test in this laboratory, and its investigation by others is recommended.

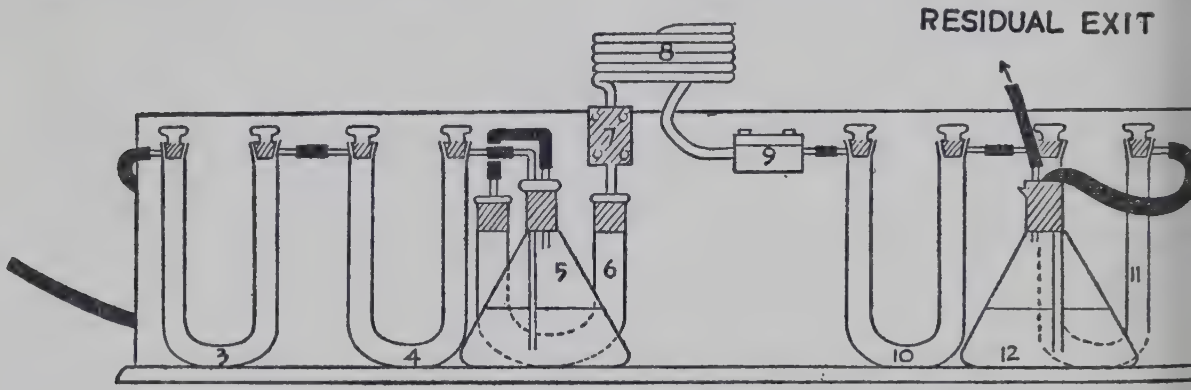
Nitrous oxide is found only in the specific case of nitrate reduction with some bacteria. The gas is very soluble in water but can be removed under reduced pressure. It is only sparingly soluble in potassium hydroxide, alkaline pyrogallol, and ammoniacal cuprous chloride. Ignited phosphorus burns vividly in the gas decomposing it and liberating nitrogen. It does not form brown fumes with nitric oxide. Burned with an electrically heated iron or nickel wire, it yields its own volume of nitrogen.

Carbon monoxide, although metaboliz by some bacteria, is not a byproduct. experiments involving this gas the e product of the oxidation is carbon dioxi The gases can be separated from each oth and from oxygen by successive absorpti in Hempel pipettes in 36 per cent potassiu hydroxide for CO_2; alkaline pyrogal (30 per cent pyrogallic acid and 60 per ce KOH mixed in the ratio of 2:7) for oxyge and freshly prepared ammoniacal cupro chloride (prepared by dissolving 11.5 g of cuprous chloride in 43 ml. of conce trated ammonia and 50 ml. of water) f carbon monoxide.

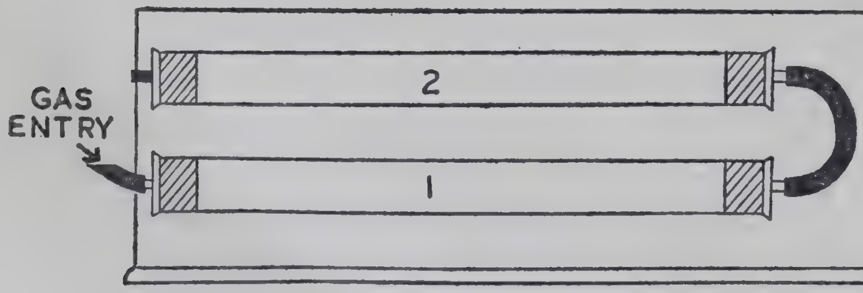
Mixtures of carbon dioxide, methar hydrogen, and hydrogen sulfide can analyzed in the following manner, adapt from a method described by Lugg.

PREPARATION OF THE SAMPLE AND APPAR TUS

The apparatus is shown in figure 4. C lect the gas sample in a large reservo Connect this reservoir and one other co taining an equal amount of pure medi



FRONT ELEVATION



BACK ELEVATION

FIG. 4

gen via individual taps to a gas manifold
nected to the gas inlet. Arrange the
rvoir so that when the taps are opened
gases will flow out at an equal rate.

lace dried silica gel in tube 1 to remove
); anhydrous $CaCl_2$ in tube 2 to remove
); Ascarite in U-tube 3 to remove CO_2 ;
Cl_2 in U-tube 4 to remove H_2S; con-
trated NaOH in flask 5 to remove HCl;
ixture of silica gel and anhydrous $CaCl_2$
U-tube 6 to remove H_2O; alternate
ers of P_2O_5 and glass wool in U-tube
to remove moisture; Ascarite in tube 11
bsorb CO_2 ; and alkaline pyrogallol in
k 12 to remove oxygen.

he inert gas which finally leaves flask
is probably nitrogen.

ry gas from tube 6 passes through
nection 7 to a copper combustion tube,
onsisting of a spiral of fine bore copper
ing 6 feet in length heated in an elec-
muffle furnace at 700° C.

THOD OF OPERATION

pen taps on U-tubes 3, 4, 10, and 11,
d flush through with O_2 . Use 1 L. of O_2 .

Make sure the temperature of the elec-
muffle is 700° C. Shut off the O_2 , and
se the taps on U-tubes 3, 4, 10, and 11.

Remove the four U-tubes 3, 4, 10, and 11
rolling back the rubber connections and
ling the U-tubes upwards. Dry with a
th, allow to equilibrate in a balance for
minutes, and weigh.

Replace the U-tubes and then flush with
additional 500 ml. of O_2 .

Close the tap from the gas manifold, re-
the reservoir with O_2 to the same level
that of the gas mixture, then open the
s of both reservoirs to the gas manifold.
en the taps on U-tubes 3, 4, 10, and 11,
d check the time and also the tempera-
re of the muffle furnace. Start the analy-
by opening the tap from the manifold.
just this tap so that the gas flow from
h reservoir is about 5 to 10 ml. per
nute.

Measure the gas volume of the mixture
fore and after each run. The gas volume can be as low as 100 ml. or as high as 500 ml. When the required amount of gas has been passed through the apparatus, shut off the sample reservoir, but allow the O_2 to keep flowing. Refill the reservoir with O_2 , and allow another 500 ml. to flow through the apparatus. Then shut off the manifold and close the taps of U-tubes 3, 4, 10, and 11. Remove the U-tubes, weigh, and note the differences in weights before and after the run.

CALCULATION

For CO_2

Amount of weight gained = amount of CO_2 passed in grams.

$$\therefore 1 \text{ gm. molecular weight of } CO_2 = 22.4 \text{ L. at N.T.P.}$$

$$44 \text{ gm. } CO_2 = 22.4 \text{ L.}$$

$$x \text{ gm. } CO_2 = ? \text{ L. at N.T.P.}$$

For H_2S

$$H_2S + CdCl_2 \rightarrow CdS + 2\ HCl$$

Here, there is a loss of weight, because of the conversion of $CdCl_2$ to the lighter CdS Then

$$\text{G.M.W. } CdCl_2 - \text{G.M.W. } CdS \equiv 22.4 \text{ L. } H_2S,$$

i.e., decrease in weight =

$$x \text{ L. } H_2S \text{ at N.T.P}$$

From this, calculate the amount of H_2S.

For CH_4 and H_2

$$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$$

$$2\ H_2 + O_2 \rightarrow 2\ H_2O$$

The amount of water gained = water due to CH_4 + water due to H_2 . The volume of CO_2 produced is equal to the volume of CH_4 oxidized. From the equation

$$44 \text{ gm. } CO_2 \equiv 22.4 \text{ L. } CH_4$$

calculate the volume of CH_4 oxidized and from this the amount of water this CH_4 would yield. The residual water must be due to the oxidation of H_2. Calculate the latter from the equation

$$18 \text{ gm. } H_2O \equiv 22.4 \text{ L. } H_2 \text{ at N.T.P.}$$

The residual gas is inert and is probably N_2.

Reproduced by permission from J. W. H. Lugg, J. Agr. Sci., *28*, 1938, 688.

For smaller quantities of gases the reader is referred to A. C. Cumming and S. A. Kay, Quantitative Chemical Analysis, Gurney and Jackson.

Glucose Phosphate Peptone Water

Peptone (Parke-Davis)	5 gm.
K_2HPO_4	5 gm.
Distilled water	1000 ml.

Steam until dissolved; filter while hot through filter paper, cool, and adjust the pH to between 7.4 and 7.6. Add 5 gm. of glucose, dispense in 4-ml. quantities in 150- by 16-mm. tubes. Sterilize at 110° C. for 25 minutes.

Reproduced by permission from F. Kauffmann, The Enterobacteriaceae, 2nd ed., Munksgaard, Copenhagen, 1954.

Glucose Yeast - Extract Tryptone Phosphate Broth

SOLUTION A

K_2HPO_4	50 gm.
KH_2PO_4	50 gm.

Make to 500 ml. with distilled water.

SOLUTION B

$MgSO_4 \cdot 7\ H_2O$	20 gm.
NaCl	1 gm.
$FeSO_4 \cdot 4\ H_2O$	1 gm.
$MnSO_4 \cdot 4\ H_2O$	1 gm.

Make to 500 ml. with distilled water.

THE MEDIUM

Glucose	10 gm.
Difco tryptone	5 gm.
Yeast extract	3 gm.
Solution A	5 ml.
Solution B	5
Distilled water	1000

Dissolve the ingredients and steriliz 110° C. for 25 minutes.

For *isolation* of *Leuconostoc*, but not subsequent culturing, add 50 ml. of tom juice or orange juice per L.

Reproduced by permission from C Pederson, personal communication. T medium is a modification of that publis by G. J. Hucker and C. S. Pederson, N State Agr. Expt. Sta. Tech. Bull. No. 1930.

Glucose Tryptic Broth

Glucose	10
Difco Bacto-tryptone	10
Distilled water	1000

Dissolve the tryptone in the water then add the glucose. Dispense the b and sterilize at 110° C. for 25 minutes.

Methyl Red Test

This test depends on the ability of organism to produce acid from glucos amounts sufficient to reduce the pH to or less and to maintain this low pH fo least 4 days. Some methyl red-nega organisms produce sufficient acid to l the pH to 4.2 but subsequently metab the acid produced and give rise to neu byproducts within the 4-day period. time interval at which the test is perfor is therefore important. Four days is quate for those enteric organisms w grow at 37° C. and for which this test been extensively used. No comparable are available for the more psychrop bacteria, but for comparative purpose similar time interval is recommended at lower optimal temperature.

In assessing the results of the test i lation to the Voges-Proskauer reaction, ganisms which, at the 4-day interval, both methyl red-positive and Voges-P kauer-positive should be incubated f longer period to determine whether plete conversion of the acid takes place

HYL RED SOLUTION

nyl red	0.1 gm.
nol (95%)	300 ml.
illed water	200 ml.

dd a few drops of the methyl red solu- to the 4-day culture in glucose-phos- e-peptone water. A red color is posi- and yellow is negative.

OTE: It has been suggested that the sphate should be omitted from the me- n for sporing bacilli, because the buff- g action of the phosphate prevents the elopment of the low pH. Nothing is ed by doing this. Mere acid production species of the genus *Bacillus* is detect- in the usual sugar fermentation tests. eliminate the phosphate is, in fact, to r the conditions of the test as normally lied, and the two methods are no longer parable.

eproduced by permission from W. M. rke and H. A. Lubs, J. Infectious Dis- es, *17*, 1915, 160.

ges-Proskauer Reaction

he test depends on the ability of the anism to produce acid from glucose and sequently to convert it to acetylmethyl- binol or 2:3-butylene glycol, both neu- substances. Upon the addition of alkali owed by vigorous shaking, both sub- ices are oxidized to diacetyl which re- with the guanidine nucleus of arginine eptone to produce a pink color. Creatine lded in the O'Meara modification of the to provide an added source of the nidine nucleus and thus accelerate the

positive test is dependent primarily on ability of the organism to bring about conversion of the acid to acetoin and ondarily on the time of incubation. All ges-Proskauer-positive organisms pro- e the acid first and hence give a positive hyl red test in the early stages of incu- ion. If the incubation period is too rt, the Voges-Proskauer test may be negative or both it and the methyl red tests positive. On longer incubation the methyl red test becomes negative and the Voges-Proskauer, strongly positive.

Notes on the time of incubation given under the methyl red test apply equally well here.

Method: Grow the organism in glucose-phosphate-peptone water for 4 days.

Original Test: Add 1 ml. of a 10 per cent solution of KOH to the culture and shake vigorously with air. The color develops slowly, and the test should be read after 18 to 24 hours. A pink fluorescence is positive; no coloration, negative.

O'Meara Modification: Add a knife point of creatine to the culture, followed by 5 ml. of 40 per cent NaOH. Shake the tube thoroughly. A pink color usually appears in about 2 minutes if positive; the development of the color may be delayed, however.

Another method in common use is *Baritt's Test.*

To the 3-ml. culture add 1 ml. of a fresh 10 per cent alcoholic solution of α-naphthol, and 1.0 ml. of a 20 per cent aqueous solution of potassium hydroxide. Shake well. A bright cherry red color appears after 5 to 15 minutes, sometimes longer, and will fade after a few hours if acetoin is present in the culture.

The O'Meara test is recommended for general use. If the Barritt method is employed, the O'Meara method should be used for comparison and both tests reported.

Reproduced by permission from R. A. Q. O'Meara, J. Pathol. Bacteriol., *34*, 1931, 401; and from M. M. Barritt, J. Pathol. Bacteriol., *42*, 1936, 441.

Loeffler's Inspissated Serum for Liquefaction Tests

Glucose	2.0 gm.
Peptone	2.0 gm.
NaCl	1.0 gm.
Tap water	200 ml.

Dissolve the ingredients and filter if necessary. Add 400 ml. of blood serum and mix. Dispense in 5-ml. amounts in 150- by 13-mm. tubes and place them in a vertical position in an inspissator. Heat to 80° C. very slowly and hold at this temperature for 6 hours to coagulate the serum. Sterilize by heating to 85° C. for 20 minutes on 3 successive days.

If the glucose-peptone water is sterilized separately, and the serum is collected and added aseptically, only the initial heating is necessary to coagulate the serum.

To detect liquefaction inoculate by stabbing and incubate for 1 to 2 weeks.

The Hydrolysis of Gelatin

This may be detected by two methods.

1. GELATIN BROTH

Add 25 per cent gelatin to meat infusion or papain digest broth. Steam until all the gelatin is dissolved. Add 80 ml. of N NaOH per L. and then adjust the pH to 7.4. Cool to 50° C. Clarify through paper pulp. Dispense in 5-ml. quantities in 160- by 13-mm. tubes and sterilize at 110° C. for 25 minutes. Do not heat above this temperature or the gelling power will be lost.

Inoculation

Inoculate by stabbing the inoculum down the centre of the tube. Incubate at 22° C. for 1 month and record the time at which liquefaction becomes evident if it occurs. For organisms which will grow at this temperature in this basal medium, this procedure is satisfactory.

Liquefaction of the gelatin occurs initially somewhere along the line of inoculation. The subsequent development varies with the organism.

For organisms which will not grow at 22° C. incubation must be carried out at the required optimum. At 37° C. the gelatin melts and liquefaction can be observed only after placing the tube in an ice bath upon removal from the incubator. If only partial liquefaction has occurred, it may not be detected.

2. GELATIN AGAR

This medium is more satisfactory mesophilic organisms. It is recommend for use with all organisms. The test limited, of course, to those which grow on the medium. Other basal me may be employed provided they do contain precipitable proteins.

Dispense 10 ml. of sterile meat infusi agar or papain digest agar into a ster petri dish.

Melt a second 10-ml. quantity of t medium and a gelatin broth (as in 1 abov Add 1 ml. of the gelatin broth to the ag Mix well and pour onto the surface of t solidified agar.

Inoculate the medium and incubate the desired temperature.

To detect hydrolysis flood the plat with a 15 per cent solution of $HgCl_2$ HCl. The unhydrolyzed gelatin forms white precipitate with the mercury sa Always compare with a control plate. So organisms hydrolyze the gelatin very pidly and may completely clear the pla in 24 hours.

Acid $HgCl_2$ Solution

$HgCl_2$	15 g
H_2O	100
HCl (concentrated)	20

Mix in the order stated and shake wel

NOTE: This is a modification of the meth of W. C. Frazier, (J. Infectious Diseas *39*, 1926, 302).

Urease Production

This test depends on the ability of t organisms to hydrolyze urea with the li eration of ammonia. The rise in pH indicated by a change in color of the in cator (phenol red) from yellow to pink.

UREASE AGAR

Sterile Urea Solution

Dissolve 20 gm. of urea in 100 ml. distilled water and sterilize by filtration.

l Medium

to-peptone	1.0 gm.
Cl	5.0 gm.
$_2PO_4$	2.0 gm.
illed water	1000 ml.

issolve the ingredients and steam for
minutes. Add 1 gm. of glucose and dis-
e by agitation. Add 0.012 gm. of phenol
and mix well. The medium should have
H of 6.8 to 6.9 and be yellow in color.

dd 20 gm. of agar and dissolve at
° C. for 20 minutes.

ool to between 55° and 60° C. in a
er bath.

Varm the sterile urea solution to the
e temperature and then add aseptically
ml. per L. of basal medium.

istribute aseptically in sterile, screw
bottles or test tubes and allow to set in
oping position.

noculate and examine daily for 7 days.

Reproduced by permission from W. B.
ristensen, J. Bacteriol., *52*, 1946, 561;
l from F. Kauffmann, The Enterobac-
iaceae, 2nd ed., Munksgaard, Copen-
gen, 1954.

drolysis of Agar

Care should be exercised in assessing this
racteristic. In agar media which contain
d particles such as bacterial cells or
ulose or chitin, bacterial attack on these
ticles may result in their digestion fol-
ed by the collapse of the agar. This
ally results in a depression in the agar
ich remains quite firm.

noculate the organism when possible on
into a clarified agar medium which sup-
ts luxuriant growth. Incubate for at
st 14 days. If surface inoculations are
de, the agar is hydrolyzed around the
lividual colonies which slowly sink into
softened medium. If agar deeps are
culated by the stabbing method, lique-
tion will progress outwards from the line
the stab.

When special media are used for cultivation, a statement regarding growth on meat infusion agar should be included for comparative purposes.

Chitin Media

PREPARATION OF CHITIN

Scrub and clean crab or lobster shells and soak them for 1 week in 1 per cent HCl; change the acid three times during this period. The acid decalcifies the shells which should become limp and leathery. Wash the shells well with water and then cut them into strips .5 to 1 inch wide.

Soak the strips in 2 per cent KOH for 10 days. During this period heat five times to a temperature just below the boiling point and then cool. This treatment extracts some pigment and removes protein and other organic matter except chitin.

Wash the strips free of alkali and extract with several changes of ethyl alcohol until all color is removed. Dry.

The strips are suitable for use in a liquid mineral salts medium for the growth of crude cultures and maintenance of chitinoclastic organisms.

For use in agar media the chitin must be finely dispersed. Take some of the strips and place them in a 3.0-L. flask. Hold 1500 ml. of cold water in readiness in a second flask. Add 100 ml. of 50 per cent H_2SO_4 to the chitin strips.

As soon as the chitin disappears, dilute the acid rapidly with the 1500 ml. of water. Allow the reprecipitated chitin to settle overnight, decant the supernatant, and wash the chitin by repeated centrifugation until neutral to litmus. Alternatively, dialyze the chitin free of acid by suspending it in a sheet of nonwater-proofed cellophane in a stream of running water.

Resuspend the chitin in water to a density such that after diluting 1:10, 5 ml. dispensed in a 10-cm. petri dish, will give a slight but distinct opalescence.

Distribute in 10-ml. quantities and sterilize at 121° C. for 15 minutes.

PREPARATION OF MEDIA

1. Chitin Strip-Mineral Salts Liquid Medium

K_2HPO_4	1.0 gm.
$MgSO_4 \cdot 7\,H_2O$	0.5 gm.
NaCl	fresh, 0.5 gm.; marine, 30 gm.
$CaCl_2 \cdot 2\,H_2O$	0.1 gm.
$FePO_4$	0.001 gm.
NH_4Cl	1.0 gm.
H_2O	1000 ml.

Dissolve the mineral salts, adjust the pH to 7.0, and distribute in bottles or test tubes. Add a strip of chitin to each container and sterilize at 121° C. for 15 minutes.

2. Chitin Agar

(*a*) Prepare a mineral salts agar base by the addition of 1.5 to 2.0 per cent agar to the mineral salts medium in 1 above. Dissolve the agar by autoclaving, adjust the pH to 7.0, and distribute in 5- and 10-ml. amounts in suitable containers. Sterilize at 121° C. for 15 minutes.

(*b*) To prepare chitin agar plates, melt one 10-ml. and one 5-ml. mineral salts agar deep and cool to 45° C. in a water bath. Pour the 10-ml. quantity into a sterile petri dish and allow to set.

To the 5-ml. quantity add 0.5 ml. of the warmed chitin suspension, mix well, and then pour as a layer on the surface of the mineral salts agar base.

This practice minimizes the time taken for chitinoclastic organisms to bring about a visible change in the suspended chitin in the medium.

ISOLATION OF CHITINOCLASTIC BACTERIA

To obtain enrichment cultures, inoculate soils or marine muds into the mineral medium containing the chitin strips. Incubate at the desired temperature until obvious decomposition has occurred. Subculture to another tube of the same medium and reincubate. When decomposition becomes rapid, inoculate the surface of a chitin agar plate, and pick off the colonies of chitin-hydrolyzing organisms. Purify in the usual manner and maintain in the liquid mediu subculture when the strips of chitin h nearly disappeared.

Reproduced by permission from L. Campbell, Jr. and O. B. Williams, J. G Microbiol., *5*, 1951, 894; and from A. Benton, J. Bacteriol., *29*, 1935, 449.

Starch Agar

The hydrolysis of starch is dependent the growth of the organism on the ba medium in which the starch is incorporat

Prepare the basal medium both in sin and double strength.

Pour a layer (15 ml.) of the sterile sin strength medium in a petri dish and al it to set.

Prepare a 10 per cent suspension of sta and bring to a boil over an open flame w continuous stirring. Autoclave at 121° for 25 minutes.

Dissolve the double strength agar ba mix with an equal quantity of the ste starch suspension, and pour as a thin la (5 ml.) over the surface of the sin strength agar base.

Refrigerate the plates for 2 days bef use. This increases the opacity.

Inoculate the medium and incubate the desired temperature. Hydrolysis m be indicated by a clearing of the mediu around or under the colony. This may checked by flooding the plate with dil iodine. Unhydrolyzed starch will turn bl

Reference: Author' Recommendation.

Alginate Agar

The composition of the following medi is, with minor modification, that describ by Waksman and his associates. T method of distribution is one found m satisfactory in the author's laboratory.

Dissolve 10 gm. of sodium alginate i L. of distilled water. Adjust the pH to with HCl and then add

$NaNO_3$	2 g
KCl	0.5 g
$MgSO_4 \cdot 7\,H_2O$	0.5 g
$FeSO_4 \cdot 6\,H_2O$	0.01 g
KH_2PO_4	1.0 g

l. . . .30 to 35 gm. (required only for
marine species)
. . . . 20 gm.

issolve the salts and the agar, and re-
st the pH to 7.0 if necessary. Sterilize
21° C. for 15 minutes.

r the preparation of agar plates, pre-
a mineral salts agar of the above com-
ion with the omission of the sodium
ate. Distribute in 10-ml. amounts in
le petri dishes and allow to set. Pour 10
f the alginate agar onto the surface of
nineral base and allow to set.

oculate the plates and incubate for 14
; examine at intervals for clarification
e agar around the colonies. Where this
rs the digestion of the alginate results
pitting of the agar which should not be
used with agar hydrolysis.

TE: The composition of the medium as
ribed restricts the growth of organisms
h do not utilize nitrate. The substitu-
of ammonium sulfate for the nitrate
commended, although for comparative
ies use of the original medium must be
le.

eproduced by permission from S. A.
ksman, C. L. Carey, and M. C. Allen,
acteriol., *28*, 1934, 213.

terial Cell Suspension Agar for obacteria

epare a suspension of the organism
ally *Escherichia coli*) of such a density
15 ml. dispensed in a 10-cm. petri dish
s a distinct opalescence. Add a quan-
of agar which will solidify but not
ssively harden the medium. Autoclave
15° C. for 20 minutes. If necessary,
st the pH and re-autoclave.

sular Polysaccharide Media Used Saccharobacterium

AL MEDIUM 1

$L_4)_2SO_4$	1 gm.
PO_4	2 gm.
water	1000 ml.

his medium has a pH of 7.4 and does
equire adjustment.

BASAL MEDIUM 2

$MgSO_4 \cdot 7\ H_2O$	0.2 gm.
$NH_4 \cdot H_2PO_4$	1.5 gm.
$CaCl_2$	0.1 gm.
KCl	0.1 gm.
$FeCl_3$	0.0001 gm.
Distilled water	1000 ml.

This medium has a pH of 7.4 and does not require adjustment.

Addition of more than 0.5 per cent NaCl, 0.3 per cent beef extract, or 0.5 per cent peptone to either medium completely inhibits growth.

The above media with a suitable carbohydrate, support the growth of the two species originally described (Sickles and Shaw, J. Bacteriol., *28*, 1934, 422).

PREPARATION OF TYPE I PNEUMOCOCCAL POLYSACCHARIDE

The organism selected for preparation of capsular polysaccharide should be freshly isolated or should have undergone several passages through the mouse.

Grow the culture in a suitable medium without neutralization during growth. The semidefined medium of Adams and Roe (J. Bacteriol., *49*, 1945, 419) is recommended. Chill the cultures in an ice-salt bath. Add an equal volume of 95 per cent alcohol chilled to 0° C. Set aside in the cold to settle. Siphon off the supernatant and centrifuge the sediment.

All the following operations must be carried out in the cold (0° to 5° C.) and should not extend over a period of more than 2 or 3 weeks.

Suspend the deposit in 80 ml. of acetate buffer (dissolve 157.5 gm. of $NaC_2H_3O_2 \cdot 3\ H_2O$ in distilled water, make up to 3 L., adjust the pH to 6.05 with glacial acetic acid, add toluene as a preservative, and store in the cold). Grind in a slowly rotating 1-L. round-bottomed Pyrex flask for 6 hours with stainless steel balls 2 mm. in diameter, and with the flask partially immersed in ice-water.

Transfer the suspension to 50-ml. plastic tubes with a little buffer and centrifuge at

8000 r.p.m. for 30 minutes. (The authors used a Servall SS-2 vacuum centrifuge). Pour off the slightly yellow viscid supernatant and precipitate the polysaccharide by the addition of 0.5 volumes of cold isopropyl alcohol (Fraction A). Take up the residue from the first grinding with 80 ml. of acetate buffer, and grind for 6 hours as before. Centrifuge and precipitate the polysaccharide from the supernatant with isopropyl alcohol (Fraction B). Discard the cell residue and treat the separate fractions as follows.

Separate the polysaccharide in a refrigerated centrifuge. Dissolve the precipitate in 100 ml. of cold distilled water (0° to 5° C.). To deproteinize the resulting greenish solution, add 50 ml. of chloroform per 150 ml. of solution and agitate intermittently in a Waring Blendor in a cold room at 3°. In order to prevent excessive warming, alternately agitate and allow to stand for 5-minute periods each.

Centrifuge the emulsion and separate the clear aqueous chloroform layers. Wash the emulsion layer with 2- by 50-ml. lots of water. Keep these washings to wash additional emulsion layers.

To the aqueous layer add an additional 50 ml. of chloroform and 10 ml. of *n*-butanol; shake and recentrifuge. A smaller emulsion layer forms. Separate the layers and rewash the emulsion layer with the previous washings.

Repeat the treatment of the aqueous layer with chloroform (and *n*-butanol?) until no emulsion layer forms.

Combine the washings from the emulsion layers and treat with fresh additions of chloroform (only?) until no emulsion layer forms. Then combine with the main aqueous layer.

Adjust the aqueous layer to a concentration of 3 per cent sodium acetate with saturated sodium acetate solution brought to pH 6.0 with acetic acid.

Add 0.5 volume of cold isopropyl alcohol to precipitate the polysaccharide and leave overnight. Repeat the procedure twice until
no middle layer of desaturated pro
forms. Centrifuge off the polysacchar
dissolve in 100 ml. of water and dial
under negative pressure in Visking
equivalent) sausage tubing with d
changes of distilled water until nega
tests for sodium phosphate and acetate
obtained.

Centrifuge in the cold at 10,000 r.p
for 30 minutes to remove any small qu
tity of sedimentable material. Lyophi
the resulting clear, colorless, highly visc
supernatant.

The above method is suitable for pr
mococcal Type I polysaccharide and r
also be suitable, with slight modificati
(see M. Heidelberger, F. E. Kendall,
H. W. Scherp, J. Exptl. Med., *64*, 1
559) for other pneumococcal types.

Reproduced by permission from
Heidelberger, C. M. MacLeod, H. Ma
witz, and A. S. Roe., J. Exptl. Med.,
1950, 341; and from R. Dubos and O
Avery, J. Exptl. Med., *54*, 1931, 51.

Carrot or Potato Plug Medium Detection of Soft Rot Organisms

Select young clean carrots (or new
tatoes) preferably more than 1.5 inche
the widest part. Scrub the surface wi
soft brush and then immerse in 0.2 per
$HgCl_2$ for 2 minutes. Handling the ca
with aseptic precautions, rinse in sev
changes of sterile tap water in a st
container. Cut across the center wit
sterilized knife. Using a sterilized 6-
cork borer fitted with a plunger, ext
several cores of carrot by plunging
borer through the cut surface as fa
possible without breaking through the
side surface. Discharge the plugs int
sterile petri dish. With a sterile knife,
move a few millimeters of the end of
core which formed the face of the orig
cut. Cut the plugs into 4- 5-cm. len
and transfer them aseptically to tube
sterile peptone water. Incubate for 3
to check sterility and store.

When testing an organism for its ab

ecompose the carrot, incubate a con- tube with the test, and at intervals k the consistency of the carrot plug in noculated tube with a stiff mounted le and compare with the control.

similar medium may be made with to.

ference: Author's recommendation.

ringa's Pectate Gel (Dowson's lification) for Detection of Soft Rot anisms

ARATION OF THE MEDIUM

alcium Agar

extract	500 ml.
l	0.5 gm.
l_2	2.5 gm.
st extract	0.5 gm.
r	10.0 gm.

issolve the ingredients and adjust the to 9.0. Autoclave at 121° C. for 15 tes. Pour enough to cover the bottom ach petri dish and place in a desiccator ry thoroughly (for about 2 days).

he 'Pectin' Solution.

repare a 2 per cent sodium polypectate tion by adding the powder slowly, with inuous stirring, to hot distilled water. 4 per cent bromothymol blue, and st the pH to 8.0 by adding N sodium oxide drop by drop. Autoclave at C. for 2 minutes. When cold pour just gh to cover the surface of the agar es and leave to set and dry in a desic- r (for about 8 days). The plates are y for streaking, if no liquid exudes from een the layers of pectate gel and agar n the plates are slightly tipped up. They well under a bell jar at laboratory perature.

OTE: Dowson (personal communication, tember, 1958) recommends the use of s sodium pectate (Product No. 24 K12) inable from Sunkist Inc., of Ontario, fornia, U. S. A. or via London from S. S. Services Ltd., 72 Victoria Street, don, S.W.1.

This product will differentiate between the pectolytic pseudomonads of Paton (Nature, *181*, 1958, No. 4601) and the pectolytic species of *Erwinia*. British pectate made from apples does not.

Reproduced by permission from W. J. Dowson, Nature, *179*, 1957, No. 4561.

Medium for *Zymobacterium (oroticum)*

Tryptone	20.0 gm.
Glucose	5.0 gm.
Yeast extract	0.5 gm.
Sodium thioglycollate	0.5 gm.
$Mg \cdot SO_4 \cdot 7\ H_2O$	0.2 gm.
$FeSO_4 \cdot 2\ H_2O$	0.005 gm.
$MnSO_4 \cdot 2\ H_2O$	0.005 gm.
$Na \cdot Mo \cdot O_4 \cdot 2\ H_2O$	0.005 gm.
Phosphate buffer, .05 M, pH 7.4	1000 ml.

Dissolve the ingredients and sterilize in sealed containers to prevent oxidation of the thioglycollate. Incubate in the presence of alkaline pyrogallol.

Reproduced by permission from J. F. Wachsman and H. A. Barker, J. Bacteriol., *68*, 1954, 400.

NOTE: The author has found it preferable to omit the thioglycollate from the medium and add it when required. Prepare a solution containing 1000 p.p.m. of thioglycollic acid in .001 N HCl. The acid solution is stable. When required add 1 ml. of the solution to 10 ml. of medium. Usually this causes no perceptible change in pH in the average medium.

Media for *Zymomonas*

Isolation Medium: Treat apple juice overnight with 1 gm. of Pectozyme (Norman, Evans, and Rais, Ltd., Manchester, England) per 100 ml. Filter and add 1 per cent Difco yeast extract. Adjust the pH to 4.5 and filter the medium again. Add agar to 3 per cent (w/v) to make a solid medium. Autoclave the medium at 115° C. for 15 minutes.

Stock Culture Medium: Maintain cultures as stabs in screw cap bottles containing apple juice diluted 1:4 with water and

1 per cent (w/v) Difco yeast extract, solidified with 10 per cent (w/v) gelatin at pH 5.5.

Reproduced by permission from N. Millis, J. Gen. Microbiol., *15*, 1956, 521; and from J. L. Shimwell, J. Inst. Brewing, *43*, 1937, 507.

Alcohol Yeast Water for *Acetobacter*

Dissolve 10 gm. of Bacto-tryptose in 1 L. of distilled water, and add 1 gm. of yeast extract and dissolve. Distribute in known quantities in small Erlenmeyer flasks to give a layer not more than 1 cm. deep. Sterilize by autoclaving at 121° C. for 20 minutes.

When cool add 10 per cent by volume of sterile absolute alcohol.

Inoculate the medium and incubate for 1 week or until obvious growth has occurred. To observe acid formation add a few drops of 1.6 per cent alcoholic solution of bromocresol purple.

NOTE: Numerous organisms may grow in this medium without oxidation of the alcohol, thus the test for acid is necessary.

Wort Agar

Use unhopped sweet beer wort, and adjust the specific gravity to 1.04. Add 2.5 per cent agar. Autoclave to dissolve, filter through paper pulp, and sterilize at 115° C. for 20 minutes.

Since the medium is at an acid pH (5.0 to 5.5), excessive heating should be avoided or the medium tends to lose its gelling power.

Media for Luminescent Bacteria

TAP WATER LEMCO AGAR

Lemco	10 gm.
Peptone (Oxoid)	10 gm.
NaCl	5 gm.
Agar	14 gm.
Tap water	1000 ml.

Dissolve the ingredients by autoclaving at 121° C. for 15 minutes. Filter if necessary. Adjust the pH to 7.6. Sterilize at 121° C. for 15 minutes.

SEA WATER LEMCO AGAR

Prepare as above but substitute 750 of aged sea water for an equal amoun the tap water.

Reproduced by permission from Spencer, J. Gen. Microbiol., *13*, 1955,

3 PER CENT NaCl FISH AGAR

To 500 gm. of fresh, minced, salt w fish add 1 L. of tap water and 10 gm peptone. Steam for 1 to 1.5 hours. F while hot through cotton wool. Add 3 40 ml. of N NaOH. Autoclave and filte the precipitate while hot. Add 30 gm NaCl and readjust the pH to between and 8.4. Add 2 per cent agar and autoc at 121° C. for 20 minutes.

Media for Halophilic Bacteria

MEDIUM A

Difco yeast extract	10.0
Proteose peptone	5.0
Casamino acids	5.0
KCl	2.0
Trisodium citrate	3.0
$MgSO_4 \cdot 7\ H_2O$	20.0
NaCl	20.0
Water	1000

Dissolve the ingredients and adjust pH to between 7.0 and 7.2.

For a solid medium add 20.0 gm. of a dissolve by heating to 121° C. for 15 r utes, filter, and adjust the medium t pH between 7.0 and 7.2. Sterilize at 121 for 15 minutes.

MEDIUM B

$MgSO_4 \cdot 7\ H_2O$	5.0
$MgNO_3 \cdot 6\ H_2O$	1.0
$FeCl_3 \cdot 7\ H_2O$	0.025
Difco proteose peptone No. 3	5.0
Glycerol	10.0
Distilled water	500

Dissolve and sterilize at 121° C. fo minutes. If agar is needed add 30.0 of it before sterilizing the medium. F if necessary.

erilize 200 gm. of NaCl in a 3- to
flask.
ld 50 gm. of skim milk powder to 500
of water and sterilize at 110° C. for 25
tes.
ld the hot agar to the NaCl, and dis-
e as much salt as possible. Allow it to
to 60° C. Warm the sterile milk to
veen 50° and 60° C., and then add the
to the salt mixture and tube or pour
tically. Care must be exercized in the
ng or the milk will curdle.
eproduced by permission from N. E.
oons, private communication. Medium
a modification of that described by N.
Gibbons, Can. J. Microbiol., *3*, 1957,
and Medium B is a modification of
published by H. P. Dussault and
Lachance, J. Fisheries Research Board
. *9*(3), 1952, 157.

bit Dung Agar for Myxobacteria

bit dung pellets	100 gm.
water	250 ml.
r	15 gm.

oil the pellets in the water for 10 min-
and stand overnight. Filter through
ze, expressing as much liquid as pos-
e. Make the volume up to 1000 ml.,
the agar, and autoclave at 115° C. for
inutes.
eproduced by permission from S. F.
szko, J. McAllister, and E. R. Hitch-
J. Bacteriol., *41*, 1941, 26; and from
I. Beebe, Iowa State Coll. J. Sci., *15*,
, 307.

pek-Dox Medium for Fungi

ium nitrate	2.0 gm.
assium chloride	0.5 gm.
gnesium sulfate	0.5 gm.
rous sulfate	0.01 gm.
assium dihydrogen phosphate	1.0 gm.
cose	50 gm.
water	1 L.

terilize at 111° C. for 25 minutes.
eproduced by permission from H.
strick and W. Rintoul, Phil. Trans.
. Soc. London, Ser. B, *220*, 1931, 7.

Ferric Ammonium Citrate Agar for the Deposition of Iron

Peptone	1.0 gm.
Yeast extract	0.2 gm.
Ferric ammonium citrate	0.3 gm.
Agar	14 gm.
Tap water	1000 ml.

Dissolve, adjust the pH to 7.0, filter if necessary, and sterilize at 121° C. for 20 minutes.

Reference: Author's recommendation.

Blood Agar

For diagnostic work a blood agar plate must be uniformly thin, free of air bubbles, and with blood cells evenly distributed. Petri dishes are rarely flat in the base. It is therefore common practice to pour a layer of saline agar into the dish to even out the imperfections in the glass and to reduce the thickness of the layer of blood agar required.

Saline Agar: Add 20 gm. of agar and 8.5 gm. of NaCl to 1000 ml. of distilled water. Steam or autoclave to melt, filter, and sterilize by autoclaving at 121° C. for 20 minutes.

Preparation of Blood Agar Plates: Melt the saline agar in a steamer, cool to 60° C., and pour it out in 10-ml. quantities into sterile petri dishes. Allow the agar to set.

Melt a bottle of sterile papain digest or meat infusion agar, and place it and the container of sterile blood in a water bath at 50° C. Allow 10 minutes for the temperatures to equilibrate. Then add aseptically 10 per cent by volume of the sterile citrated blood (horse, ox, sheep, or human as desired), and mix uniformly by slowly inverting the bottle several times. *Do not shake* since frothing must be avoided.

While the blood agar is still warm, pour it in a thin layer over the saline agar.

Allow the plates to set, and incubate for 24 hours at 37° C. to check for sterility. Then refrigerate at 4°.

Bordet-Gengou Potato Glycerol Blood Agar

1. Preparation of Potato Extract: Clean and peel potatoes. Weigh 250 gm. and cut into thin slices. Add 9 gm. of NaCl and 500 ml. of water. Boil until the potato disintegrates. Filter and adjust the pH to 7.0.

2. Preparation of the Medium: Dissolve 60 gm. of agar in 1500 ml. of water by autoclaving. While still hot, add 500 ml. of heated potato extract, 20 ml. of glycerol, and 20 gm. of Difco proteose peptone. Distribute as required. To sterilize, steam for 1 hour and then autoclave at 110° C. pressure for 5 minutes.

To prepare plates melt the agar and cool to 55° C. Add 15 per cent by volume of sterile defibrinated horse (or sheep) blood previously warmed to 45°. Mix well and pour.

Reproduced by permission from T. J. Mackie and J. E. McCartney, Handbook of Practical Bacteriology, E. and S. Livingstone, Edinburgh, 1942.

Liver Extract Agar

Minced beef liver	500 gm.
Distilled water	1000 ml.

Steam for 1 hour. Adjust the pH to 7.0, and steam for an additional 10 minutes. Pressure filter and then add:

Bacto-tryptone	10 gm.
K_2HPO_4	1 gm.
Agar	15 gm.

Steam to dissolve. Adjust the pH to 7.0. Dispense and sterilize at 121° C. for 20 minutes.

Reproduced by permission from E. J. Cameron, J. Assoc. Offic. Agr. Chemists, *19*, 1936, 433.

Embryonic Egg Yolk Agar for *Calymmatobacterium*

Withdraw the yolk aseptically from 4- or 5-day-old chick embryos, and add it to an equal quantity of melted meat infusion agar held at 60° C. in 150- by 16-mm. tubes. Allow to set in a slanting position.

Inoculate the condensation water.

Reproduced by permission from K.
derson, W. A. DeMonbreun, and E.
Goodpasture, J. Exptl. Med., *81*, 1945,

Levinthal Media for the Differentiat
of the Genus *Haemophilus*

"Levinthal Stock": Prepare Difco Br
Heart Infusion Broth according to di
tions.

While it is boiling vigorously, add
per cent by volume of defibrinated h
blood. Filter through Whatman No.
filter paper, and sterilize the filtrate
filtration.

"Levinthal Agar": Dissolve 45 gm.
Difco proteose agar No. 3 and 15 gm
Bacto-agar in 1000 ml. of water. Add 1
ml. of sterile "Levinthal Stock."

"Levinthal Agar Plus X-factor": "Le
thal agar" autoclaved at 121° C. for
minutes is the "X-factor" medium.
X-factor (hemin of the blood) is stable.
V-factor is destroyed.

"V-factor" Medium: Emulsify 100 gm
powdered brewers' yeast in 400 ml.
distilled water. Adjust the pH to 4.6.
for 10 minutes. Filter through filter pa
Adjust the pH to 7.0, and sterilize by
tration.

To prepare *"V-factor"* medium ad
part of yeast extract to 9 parts of me
Difco proteose agar No. 3.

"X- plus V-factor medium": Add 1
of sterile yeast extract (see V-factor
dium) to 9 parts of "Levinthal agar
X-factor."

Reference: H. E. Alexander in R. J.
bos, Bacterial and Mycotic Infections
Man, 1st ed., J. B. Lippincott, Phila
phia, 1948, p. 484. Reproduced by per
sion of the National Foundation,
York.

Blood Cystine Glucose Agar for
teurella tularensis

Beef infusion	1000
Peptone	10

	15 gm.
um chloride	5 gm.
ne	1 gm.
se	10 gm.
rinated blood	5 to 8%

move all fat from lean beef and grind neat finely. To each 500 gm. of lean add 1000 ml. of distilled water and e in the refrigerator for 12 to 18 hours. refrigeration, skim off all fat and boil ously for 30 minutes. Filter through r and restore the volume with distilled r. Add the peptone, agar, and sodium ide. Dissolve and adjust the pH to Sterilize by autoclaving at 121° C. for inutes. When required for use, add cystine and glucose, and steam for 1 on 3 successive days to melt the agar to sterilize. Cool to 60° C. and add the d.

eat the flask of blood-glucose-cystine in a water bath for 2 hours at 60° C., care to avoid a higher temperature h will cause sedimentation. Use this to the plates.

eproduced by permission from D. T. h and D. S. Martin. Zinsser's Textbook acteriology, 9th ed., Appleton-Century-ts, New York, 1948.

hosphothiamine Medium for *Hae- hilus piscium*

proteose peptone No. 3	20 gm.
ose	10 gm.
l	5 gm.
en 40	0.05 gm.
illed water	1000 ml.

issolve the ingredients, and adjust the to 7.3 with NaOH. Sterilize by autoing at 121° C. for 15 minutes.

dd diphosphothiamine aseptically to basal medium at the rate of 1.0 μg. of iosphothiamine per ml. of basal men, *or* add adenosine triphosphate at the of 30 μg. per ml. of basal medium.

eproduced by permission from P. J. fin, Arch. Biochem., *30*, 1951, 100, demic Press Inc.

The Isolation of *Rhizobium* Spp. from Legume Nodules

ISOLATION FROM THE NODULE

Carefully soak the ground around the plant for 24 hours before removal. Remove the plant with the soil, and place it in a container filled with water to allow the soil to fall away. Wash the roots carefully in several changes of sterile tap water, and then cut off the nodules—preferably healthy ones—from the crown roots with a small piece of root adhering to each. Immerse successively for 2-minute periods in 70 per cent ethanol and 0.2 per cent mercuric chloride; then transfer aseptically to sterile water and rinse with at least three changes of water.

Crush the nodules between two sterilized slides, and inoculate some of the milky fluid onto legume extract agar or yeast extract mannitol agar. Incubate at 28° C. for several days. Subculture the colonies to slants of the same medium and incubate.

INOCULATION OF SEED

For small seeds the method of Fåhraeus (*vide infra*) is excellent and yields quick results. For large seeds the following method may be used.

Prepare carbon- and nitrogen-free agar and dispense in vessels compatible with the species of the plant being tested.

Sterilize the seeds in the same manner as the nodules. Place the seeds in a sterile petri dish or other container over a layer of yeast mannitol or legume extract agar. Incubate at 28° C. until germination occurs. Select sterile germinated seeds, immerse them in a suspension of the organism, and then transfer them to the surface of the carbon- and nitrogen-free agar. Expose the containers to daylight, but protect the seeds from direct sunlight. If the culture is a species of *Rhizobium*, nodules will begin to form shortly after the emergence of the first true leaves.

THE FÅHRAEUS PROCEDURE FOR SMALL LEGUMES

Dispense Fåhreus carbon- and nitrogen-free liquid medium in 25-ml. amounts in 150- by 39-mm. tubes, and cover with loose glass caps or plug with cotton wool.

Sterilize some clean microscope slides and 24- by 40-mm. coverglasses at 160° C. in a petri dish; place only one slide and one coverglass in each dish.

Sterilize the seeds as above and allow them to germinate at room temperature in a shallow layer of water.

When the roots are 10 to 15 mm. long, pipette approximately 0.2 ml. of the carbon- and nitrogen-free medium (containing 0.3 to 0.4 per cent Bacto agar), melted and cooled to 30° C., to one-half of a slide and immediately transfer a seedling to the slide with a sterile platinum wire so that the root tip is in the agar. Place the coverglass carefully over the agar and the root tip with forceps. Remove the seed coat if it still adheres to the cotyledons.

Transfer the slide to the tube containing the mineral medium.

Inoculate the roots either by adding the inoculum to the agar before preparing the slide or by pipetting a few drops of a heavy suspension to the mineral medium. Incubate at 25° C. in a controlled temperature cabinet under a fluorescent lamp.

To examine, remove the slides and drain off the excess solution with a filter paper. Examine under high power with the use of glycerol as an immersion fluid. Slides may then be returned to the tubes. A phase contrast microscope should be used if available.

Infection of the root hairs coincides, in clover, with the opening of the first true leaves. It is preceded by intense protoplasmic activity and a movement of the cell nucleus to the point of infection. Bulbous swelling and curling of the root hairs are common features. Infection threads are visible within the root hair. Unaffected hairs usually remain straight and normal in form.

Reference should be made to the original paper for a more detailed descriptio
for the excellent illustrations.

FÅHRAEUS CARBON- AND NITROGEN- LIQUID MEDIUM

$CaCl_2$	0.
$MgSO_4 \cdot 7\ H_2O$	0.1
KH_2PO_4	0.
$Na_2HPO_4 \cdot 2\ H_2O$	0.1
Fe citrate	0.00
Mn·Cu·Zn·B·Mo	T
Distilled water	100

After autoclaving the pH is 6.5.

For the agar medium add 0.3 to 0.
cent agar before sterilization.

Sterilize at 121° C. for 20 minutes.

Reproduced by permission from
Fåhraeus, J. Gen. Microbiol., *16*, 1957

Legume Extract Agar for *Rhizo*

Cut up 35 gm. of well washed a
roots and mix with 10 gm. of soybean
Add 3 times the volume of water,
gently for 1 hour, and then allow to
overnight. Dilute to 1000 ml. and
through paper pulp. Add the followi

K_2HPO_4	1.
$MgSO_4 \cdot 7\ H_2O$	0.
NaCl	0.
$CaCl_2$	0.
$FeCl_3$	0.001
Agar	20.

Heat to 121° C. for 20 minutes, filte
add:

$CaCO_3$	
Sucrose	1
Glucose	

When the sugars have dissolved, dis
as required and sterilize at 115° C. f
minutes.

Reproduced by permission from
Pittman, J. Dept. Agr., W. Australia
2nd Ser., 1935, 105.

Yeast-Extract Mannitol Agar (Med "79")

Mannitol	10.
K_2HPO_4	0.5
$MgSO_4 \cdot 7\ H_2O$	0.2

	0.1 gm.
$)_3$	3.0 gm.
t extract (10%)*	100.0 ml.
	15.0 gm.
lled water	900.0 ml.

o prepare the yeast extract: Steam 100 of starch-free pressed yeast for 3 to 4 s in 1000 ml. of water. Allow to stand week. During this time the yeast cells settle to the bottom. The clear, straw-ed supernatant liquid should be si-ed, bottled in 100-ml. quantities, and lized at 15 pounds of pressure for 45 tes.

ch 100 ml. of the yeast extract con-s 50 to 60 mg. of amino acid nitrogen. eproduced by permission from O. N. n, Experiments in Soil Bacteriology, gess Publishing Company, Minneapo-949.

d Medium for *Leptospira*

ptose-phosphate broth (Difco)	0.2 gm.
r (Difco)	1.0 gm.

issolve in 90 ml. of distilled water, ad- the pH to 7.5, and sterilize the medium he autoclave. Cool to 50° C., and add tically 10 ml. of sterile rabbit serum 1 ml. of hemoglobin preparation. Heat 6° for 30 minutes and then pour plates. repare the hemoglobin preparation by ng washed and packed sheep erythro-s in 20 volumes of cold distilled water. nove the stroma by centrifugation. ilize by Seitz filtration.

eproduced by permission from C. D. and A. D. Larson, J. Bacteriol., *73*, 7, 587.

See also J. Bacteriol., *73*, 1957, 587.)

rmate Medium for *Methanococcus*

dium formate	15.0 gm.
nmonium sulfate	1.0 gm.
$Cl_2 \cdot 2\ H_2O$	0.01 gm.
$Cl_2 \cdot 2\ H_2O$	0.01 gm.
$Cl_3 \cdot 6\ H_2O$	0.02 gm.
$SO_4 \cdot 4\ H_2O$	0.01 gm.
$_2MoO_4 \cdot 2\ H_2O$	0.001 gm.
HPO_4	2.0 gm.
Phenol red indicator	0.003 gm.
Methylene blue	0.002 gm.
Sodium thioglycollate	0.5 gm.
Glass-distilled water	1000 ml.

For a solid medium add 1.5 gm. of agar.

If a pyrogallol-K_2CO_3 seal is used, the pH of the medium after addition of the inoculum should be 8.3 to 8.5. When an "oxsorbent" seal is employed, the medium after inoculation is adjusted to a pH between 7.5 and 8.0. Cultures are incubated at 30° to 40° C., anaerobically.

Sterilize at 121° C. for 15 minutes.

Reproduced by permission from T. C. Stadtman and H. A. Barker, J. Bacteriol., *62*, 1951, 269.

Glycine Medium for *Peptococcus glycinophilus*

Glycine	3.0 gm.
Bacto-yeast extract	5.0 gm.
Bacto-peptone	5.0 gm.
M Phosphate buffer, pH 7.1	5 ml.
Saturated $CaSO_4$	2.5 ml.
Cysteine HCl	0.2 gm.
$MgSO_4 \cdot 7\ H_2O$	0.05 gm.
$FeSO_4 \cdot 7\ H_2O$	0.01 gm.
Distilled water	1000 ml.

Dissolve the ingredients, adjust the pH to 7.2, and dispense in screw-capped bottles. Sterilize at 121° C. for 15 minutes.

Since cysteine is rapidly oxidized, the medium has a limited life. Do not expose it unnecessarily to air.

Reproduced by permission from B. P. Cardon and H. A. Barker, J. Bacteriol., *52*, 1946, 629.

Isolation and Growth of *Methanobacterium*

AN ENRICHMENT MEDIUM FOR *Methanobacterium*

Barker and Taha Enrichment Medium

Ethanol	10.0 ml.
K_2HPO_4	5.0 gm.
$MgSO_4 \cdot 7\ H_2O$	0.1 gm.
$(NH_4)_2SO_4$	0.3 gm.
$FeSO_4 \cdot 7\ H_2O$	0.02 gm.

Yeast autolysate	5.0 ml.
$CaCO_3$	100 gm.
Water	1000 ml.

Sterilize the above medium by autoclaving.

Prepare separately (*a*) 0.5 gm. of Na_2CO_3 and 10.0 ml. of H_2O, and (*b*) 0.1 gm. of $Na_2S \cdot 9\ H_2O$ and 10.0 ml. of H_2O. Sterilize both by autoclaving, and then add aseptically to the above medium. Adjust the pH to between 7.0 and 7.4.

A similar medium containing 15 to 20 gm. of agar per L. may be used for isolation.

NOTE: The ethanol is included in the above basal medium before autoclaving. As an alternative it may be sterilized by filtration and added aseptically with the Na_2CO_3 and $Na_2S \cdot 9\ H_2O$.

ISOLATION

Dispense the enrichment medium in glass-stoppered bottles in which a heavy (5 per cent) inoculum of black swamp mud has been placed. Fill to capacity to exclude air and insert stopper. Incubate at 35° C. in the dark. *Methanobacterium* usually develops in a few days as the predominant population. Transfer through the enrichment medium two or three times, and then isolate by the streak plate or shake tube method.

Reproduced by permission from H. A. Barker and S. M. Taha, J. Bacteriol., *43*, 1942, 347.

Isolation and Growth of *Dialister* Sp.

Source of the Organisms: The organisms, obtained from nasopharyngeal washings from patients with epidemic influenza, were originally described by Olitzky and Gates under the name of *Bacterium pneumosintes*. Because of their small size, the organisms pass through the Berkefeld N- and V-filters, and filtered material may be used. The organisms also survive prolonged contact with 50 per cent glycerol which kills many other bacteria. No growth occurs below pH 7.0 or above pH 8.0.

Smith-Noguchi Medium and Technique for Isolation: Place relatively large p (0.6 to 0.8 gm.) of sterile normal ra kidney in sterile test tubes measurin by 1.5 cm., one piece to each tube.

Cover the tissue with 8 to 10 m sterile human ascitic fluid at pH 7.8 to Seal each tube with 2 ml. of sterile m paraffin and replace the cotton wool

Incubate for 2 days at 37° C. to c sterility and permit anaerobic conditio develop.

Inoculation: Nasopharyngeal fluid be inoculated directly or after filtra through a Berkefeld V- or N- or equiva filter. Suspected tissue should be macer in saline.

Inoculate the medium with a capi pipette pushed through the paraffin. posit the inoculum on the surface of meat. Reseal by applying a heated ro the surface of the paraffin.

Incubate at 37° C. for 8 to 12 days. positive cultures a faint haze appear 5 days and reaches a depth of 3 cm. by 8th day.

Subculture to blood agar, Loeffler's spissated serum, or Bordet-Gengou med and incubate anaerobically. Growth oc within 7 days at 37° C.

Reproduced by permission from P. Olitzky and F. L. Gates, J. Exptl. Med. 1921, 713.

Isolation and Cultivation of *De fovibrio*

Desulfovibrio desulfuricans is a s anaerobe noted for its ability to re sulfates. It occurs in soil and marine m and is of common occurrence in se sludge. The following methods are of Butlin and his associates and of M with modifications suggested by Gross and Postgate.

MEDIA FOR ISOLATION OF *Desulfovibrio*

The following modifications of m described by Baars (1930) and Sta (1938) are recommended by Butlin, Ad and Thomas.

ars' Medium

O_4	0.5 gm.
l	1.0 gm.
4	1.0 gm.
$_4 \cdot 7\ H_2O$	2.0 gm.
ctate, 70% solution	5.0 gm.
vater	1000 ml.

solve the salts; adjust the pH to n the range of 7.0 to 7.5 and sterilize 1° C. for 20 minutes

pare separately a 1 per cent solution $SO_4 \cdot (NH_4)_2SO_4 \cdot 6\ H_2O$ and, sterilize teaming for 1 hour on 3 successive Add 5 ml. of the supernatant per 100 f the above medium immediately be- use.

TE: This medium has a heavy precipi- but is quite suited for crude cultures.

arkey's Medium

PO_4	0.5 gm.
Cl	1.0 gm.
O_4	1.0 gm.
$l_2 \cdot 2\ H_2O$	0.1 gm.
$O_4 \cdot 7\ H_2O$	2.0 gm.
actate, 70% solution	5.0 gm.
illed water	1000 ml.

issolve the ingredients and adjust the to between 7.0 and 7.5. Sterilize at C. for 20 minutes.

TE: This medium has a slight precipi- which may be removed by filtration sterilization following which the me- n may be resterilized.

epare a 1 per cent solution of $FeSO_4 \cdot$ $_4)_2SO_4 \cdot 6\ H_2O$ as for 1 above, and add 5 per 100 ml. of medium just before use.

r *halophilic strains* add 1 to 3 per NaCl to each of the above media be- sterilization, or, alternatively, replace tap or distilled water with sea water.

ELOPMENT OF CRUDE CULTURES

utlin and his associates recommend the of the above media with *and without* addition of sodium sulfite for prepara- of enrichment cultures. The sodium te, in concentrations up to 5 per cent, has little effect on the majority of sulfate reducers but materially reduces the development of the nonsulfate reducers.

Prepare a fresh 20 to 30 per cent solution of $Na_2SO_3 \cdot 7\ H_2O$ shortly before use and sterilize by filtration. When required add 10 ml. of the solution to each 100 ml. of medium, and readjust the pH to 7.2 by the addition of sterile N HCl (approximately 2 ml. per 100 ml. of Baars' medium).

Place the inoculum in two 30- to 50-ml. stoppered bottles. One to 2 gm. of soil or sewage sludge is usually adequate. Fill the bottles completely with the media (one with and one without sulfite) and insert the stopper. Incubate at 30° to 50° C.

Growth is usually indicated by the precipitation of black iron sulfide following the reduction of the sulfate by the growing cells. This may take a few days to several weeks. Examine the cultures for vibrios. If they predominate proceed to the isolation of pure cultures. If not, make subcultures from the black sludge into a fresh medium with the use of a 10 per cent inoculum.

ISOLATION OF PURE CULTURES

Prepare Starkey's medium with the addition of 2 per cent agar and filter clear. Sterilize the medium, add the ferrous salt, and then distribute aseptically in 9-ml. amounts into tubes held in a water bath at 45 to 50° C.

To one series add 1 ml. of 30 per cent sterile $Na_2SO_3 \cdot 7\ H_2O$, and 0.2 ml. of sterile N HCl.

Prepare decimal dilutions of the crude cultures. Although not specifically recommended by Butlin and his associates it is advisable to prepare the dilutions in the liquid medium in the absence of oxygen. The addition of 1 ml. of a sterile solution containing 1000 p.p.m. of thioglycollic acid to each 9 ml. of sterile liquid medium is recommended.

Add 1 ml. of each of the higher dilutions to corresponding tubes of the agar medium. Mix well and allow to set.

Incubate until well isolated black colonies appear in the higher dilutions.

Alternative: Inoculate the crude culture onto the surface of the agar medium in a petri dish, with the use of both sulfite-containing and sulfite-free media. Make the transfer preferably in an oxygen-free atmosphere, and seal the dishes in a suitable anaerobic jar without delay.

SUBCULTURE OF ISOLATED COLONIES

The foregoing media are not the most suitable for the culture of species of *Desulfovibrio.* Addition of yeast extract greatly stimulates growth but is omitted from the previous media because it also greatly stimulates contaminants. The following medium described by Butlin and his associates and modified by Postgate should be used. The medium is similar in most respects to that published independently by Miller (1950).

K_2HPO_4	0.5 gm.
NH_4Cl	1.0 gm.
Na_2SO_4	1.0 gm.
$CaCl_2 \cdot 6\ H_2O$	0.1 gm.
$MgSO_4 \cdot 7\ H_2O$	2.0 gm.
Na lactate, 70% solution	3.5 gm.
Difco yeast extract	1.0 gm.
$FeSO_4 \cdot 7\ H_2O$	0.002 gm.
Distilled water	1000 ml.

Dissolve the ingredients, adjust the pH to 7.5, and autoclave at 121° C. for 20 minutes. Filter off the sediment, dispense as required, and resterilize.

Prepare separately a 0.6 per cent solution of cysteine hydrochloride in distilled water, and sterilize by autoclaving at 121° C. for 20 minutes. This acid solution has a pH of 1.8 and is relatively stable to oxidation provided it is not neutralized. Add 1 ml. to each 9 ml. of medium immediately before use. The final concentration of cysteine is 5 μmoles per ml.

Pick black colonies showing the correct morphological types on microscopic examination into the liquid medium. Incubate aerobically.

If it is desired, the cultures may be plated on the same medium containing 2
per cent agar and incubated anaero
in an atmosphere of hydrogen and
cent CO_2 in a McIntosh and Fild
with a dried pad of absorbent cotton
impregnated with lead acetate, betwe
cultures and the catalyst.

NOTE: Reference should also be m
Grossman and Postgate, Proc. Soc.
Bacteriol., *16*, 1953, 1; and to Drum
and Postgate, J. Appl. Bacteriol., *18*,
307.

Reproduced by permission from
Butlin, M. E. Adams, and M. Thom
Gen. Microbiol., *3*, 1949, 46; J. P.
man and J. R. Postgate, Nature, *17*
600; and from L. P. Miller, Contrib.
Thompson Inst., *6* (3), 1950, 85.

Bile Solubility Test

This test has been employed to dif
tiate the pneumococcus, *Diplococcus*
moniae, from the other α hemolytic st
cocci. Living cultures of this organis
soluble in bile salts at neutral pH.

Centrifuge the growth from a 5-m
ture of the organism in glucose-t
broth, and resuspend the growth in
phosphate buffer, pH 7 to 7.6, cont
2 per cent sodium chloride and 0.0
cent sodium desoxycholate. Incuba
37° C. for 60 minutes and examine.
tures of pneumococci lyse under thes
ditions.

Reproduced by permission from
Anderson and P. D'A. Hart, Lanc
1934, 359.

Detection of Cholesterol in Cultu *Streptobacillus*

Macrotechnique: Grow the organi
20 per cent serum broth. Centrifug
wash the deposit three times with

Extract the aqueous residue cont
the organisms with acetone twice an
tane three times. Mix the acetone an
tane washings, and then separate the
ous layer and discard it. Dry the s
over anhydrous sodium sulfate, and
evaporate to dryness with complet
the final stage under reduced pressu

olve the waxy residue in N alcoholic
and reflux for 1 hour. Cool and di-
ith water. Extract thoroughly with
e.

sh the pentane solution with dilute
n hydroxide and then with distilled
Dry the pentane solution and then
rate to dryness.

rystallized white residue is left. Dis-
in 90 per cent aqueous acetone and
tallize. Flat plates of cholesterol with
lting point of 147 to 147.5° C. are
ced.

produced by permission from S. M.
idge and E. Kleineberger, J. Pathol.
riol., *52*, 1941, 219.

onin Test for Cholesterol

.5 per cent solution of digitonin in 50
ent alcohol will precipitate cholesterol
solution.

lase Production

talase is an enzyme containing a
tin as the prosthetic group and is
ble of decomposing hydrogen peroxide
seous oxygen and water. It is widely
ibuted in nature and is present in most
ic cells. The function of catalase is to
ve the toxic product H_2O_2 resulting
coupled oxidation-reduction processes
ving oxygen.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

ur 1 ml. of H_2O_2 (10 volumes per cent)
the surface of a 24-hour agar slope
re. If catalase is present bubbles of
en will be released from the surface
e growth.

eproduced by permission from W. W.
Topley and G. S. Wilson, Principles of
teriology and Immunity, 1st ed., Ed-
d Arnold and Company, London, 1929,
39.

Oxidase Test

our a freshly prepared 1 per cent aque-
solution of di- or tetramethyl-*p*-phen-
ediamine hydrochloride over the culture after 24 hours of incubation. If oxidase is produced the colonies turn bluish purple.

Reproduced by permission from J. Gordon and J. W. McLeod, J. Pathol. Bacteriol., *31*, 1928, 185.

Indole Production

The medium employed for this test must contain an adequate amount of tryptophane. Papain digest broth (p. 000) is excellent but may not be suitable for some organisms. The addition of 1.0 per cent casein digest to a suitable growth medium usually suffices.

Incubate the cultures for a minimum of 1 to 3 days, and test at 24-hour intervals on a sample of the culture.

Procedure: Layer 1 to 2 ml. of the Ehrlich-Böhme Solution No. 1 on the surface of the liquid culture. A red color develops at the junction if indole is present. The author finds that no advantage is gained by the addition of Solution No. 2 as recommended in many texts.

Preparation of solutions:

Solution 1:

para-dimethylaminobenzaldehyde	4 gm.
96 per cent ethanol	380 gm.
concentrated HCl	80 gm.

Solution 2:

Saturated aqueous solution of potassium persulfate

Goré's Modification: Remove the plug (which must be of white absorbent cotton wool) from the tube, and moisten with 0.5 ml. of Solution No. 2 and then with 0.5 ml. of Solution No. 1. Replace the plug and push it down to within 1 inch of the medium. Place the tube in a boiling water bath for 15 minutes; avoid any direct contact between the plug and the medium.

A red color on the plug indicates indole.

The method differentiates between indole and methyl-indole, since the latter is nonvolatile.

Reproduced by permission from S. N. Goré, Indian J. Med. Res., *8*, 1921, 505.

Slide Culture Technique for the Examination of Microcolonies

For this specially cleaned slides and clarified agar are used.

Clean and bake new slides in a 400° C. oven for 15 minutes to remove grease (it is quite safe to place cold slides directly in the oven). They are then ready for use.

Clarified Agar: Removal of dead bacterial cells from commercial agar is essential. A 2 per cent agar solution containing the required nutrients is used. Cut a piece of closely woven cloth the size of the base of a Buchner funnel and place it in the funnel. Put about 20 gm. of Super-Cel in a liter of hot water, stir, and then pour into the funnel while suction is applied. Empty and rinse the flask. Melt the agar solution; cool to between 50° and 60° C., and add horse serum (3 ml. per L.). Heat again, with continuous stirring until the serum coagulates, and then add 20 gm. of Super-Cel to the agar solution and pour through the pad. Repeat if microscopic examination shows that bacterial cells are still present.

Dispense in 20-ml. amounts at 121° C. and autoclave for 15 minutes.

To prepare slides, melt and pour a cl
fied agar deep into a petri dish placed o
hot plate (60° C.). Submerge sterile sli
into the agar, drain slightly, and allow
set in a sterile moist chamber.

To prepare a suitable chamber, plac
circular filter paper in the base of a pe
dish and insert two glass rods to supp
the slides. Moisten the paper and autocla
Add sterile water to the paper as requi
to keep it moist during incubation.

Preparation of Inoculum: Suspend t
cells to a barely perceptible density in a
suitable suspending fluid in a 13-mm.
ameter tube. Place 1 drop on a test ag
slide and carefully spread over the surfa
with a small sterile spreader made of th
polythene film fixed to the end of a gla
rod. To prepare a spreader cut out a pie
of thin polythene film as illustrated
figure 5.

Heat a piece of 3-mm. glass rod in a flar
until it is hot enough to adhere to the pol
thene film on contact. Roll it on the na
row end of the film as indicated.

Sterilize the spreaders by immersion
sterile 70 per cent alcohol for 10 to 15 mi
utes before use.

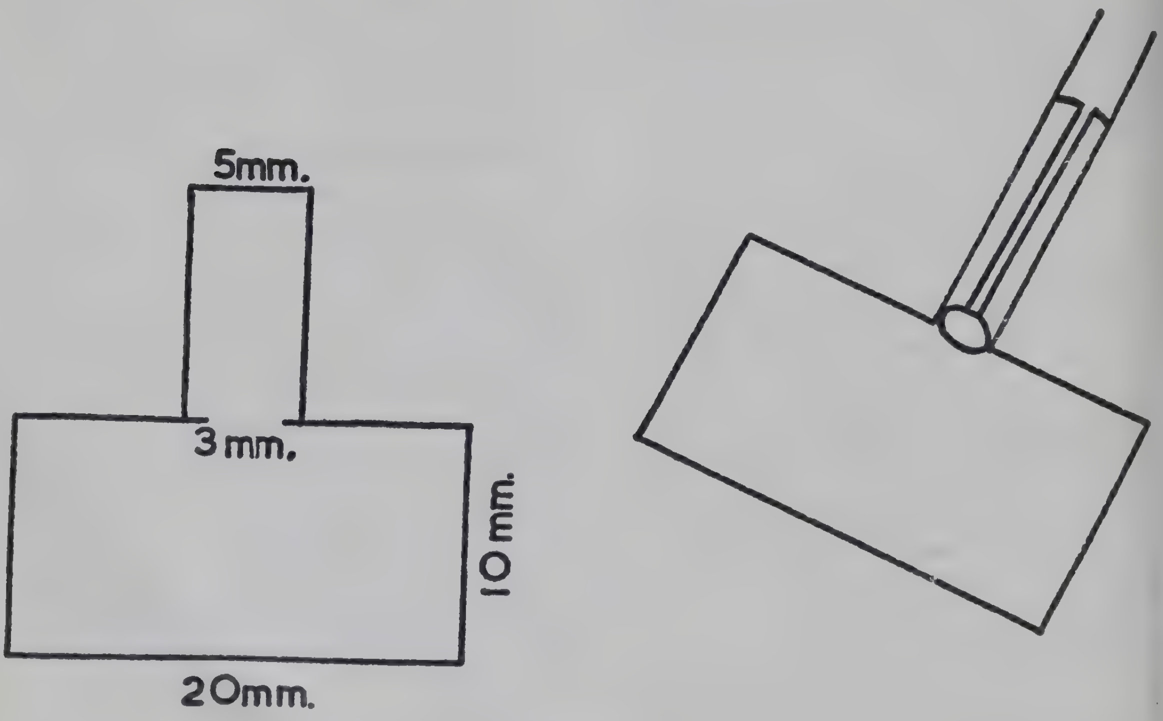


FIG. 5

is most important to obtain a uniform
ad and not to injure the agar surface.
the slide immediately in an oven at
C. Stain with filtered dilute carbol-
ısin and cover with a coverglass while
wet. Examine under oil immersion and
nt the average number of cells per field.
ute the suspension so that the count will
reduced to not more than 1 cell per field,
use for the preparation of slide cultures
the same general technique.

ncubate in the humid chambers and re-
ve slides at predetermined time inter-
s, depending on the rate of growth. Re-
ve the agar from the under side of the
le, and then dry at 105° C., stain, and
mine. If desired, a coverslip may be ap-
ed directly to the moist agar culture
hout addition of mounting fluid and the
ture examined with a phase contrast
croscope.

Reference: Author's recommendation.

easurement of Organisms

ptical Instruments for Measurement of
ganisms: For this purpose an "ocular
le" is usually employed. It consists of a
cular glass disc which is placed on the
atform inside the ocular of the micro-
pe. Etched on the surface is a scale of 50
iform divisions. The linear value for each
vision depends on the ocular magnifica-
n, draw tube length, and objective mag-
ication, and hence the scale is left un-
mbered. For use in measurement the
le must be calibrated for each combina-
n of ocular, objective, and draw tube
gth.

Calibration is done with the aid of a
tage micrometer"—a glass slide in the
nter of which is usually an accurately
librated scale 1 mm. long and divided
to .1 and .01 mm. The stage micrometer
placed on the stage of the microscope
d the ocular scale into the ocular. The
jective lens in use is then focused care-
lly on the slide scale, and the number of
age micrometer divisions (.01 mm.) cor-
sponding to the 50 divisions of the ocular
scale is determined. Thus if 50 ocular divisions = 10 scale divisions, 1 ocular division = 10/50 × .01 mm. which = .002 mm. or 2 μ (mu).

It is important that the combination of ocular magnification, objective, and draw tube length be recorded for each calibration.

Once the calibration has been done the stage micrometer is replaced with the preparation and the size of the organisms is estimated.

Measurement with Polystyrene Particles: Polystyrene particles prepared by the Dow Chemical Company have proved most useful for measurement of cells. Prepare a dilute suspension of the particles in water and use in place of water in the preparation of smears. Allow the smears to air-dry, and then cover with filtered dilute aqueous safranin (see Gram stain) and mount wet under a coverslip. The particles do not stain and can be used to measure the size of the stained cells.

The 0.8 μ particles are the most useful size for general purposes.

Reference: Author's recommendation.

Preparation of "Hanging Drops" for Motility Tests

Match Stick Technique: Carefully clean a slide and put in a clean place on the bench.

Remove the head from a "safety" match and cut the match stick in two.

Adhere the two sticks to the slide with a small daub of grease; placing them sufficiently close together to support a No. 1 square coverglass by its outer edges.

Remove any adhering dirt from a coverglass with a clean cloth and place it on the bench.

Use the 4-mm. loop, transfer a loopful of the broth culture aseptically to the center of the coverglass and then sterilize the loop.

Place a small daub of grease on the top of each match stick, and then invert the slide over the coverglass and press down gently until the coverglass adheres to the sticks.

Revert the slide quickly but carefully. The drop should remain suspended from the coverglass.

Examination of Hanging Drop Preparation for Motility: Place the preparation on the stage with the drop over the center of the condenser.

Close the iris diaphragm completely; then open slightly. With low power, focus with the coarse adjustment until the drop comes into focus (See Note 2 below). It may be necessary to open the iris diaphragm slightly.

Move the edge of the drop to the center of the field; focus with the fine adjustment and then swing in high power. (See Note 1 below).

Adjust the light and focus with the fine adjustment until the drop is again in focus. Organisms should be seen either swimming or motionless near the edge of the drop.

For an organism to be considered motile, at least *one* organism should be seen to alter its position in relation to at least two other organisms. It is *not* necessary for all organisms in a drop prepared from a *pure culture* to be in motion. Nonmotile organisms either all remain stationary or exhibit Brownian movement.

NOTE: 1. Not all microscopes are so constructed as to allow the high power objective to be swung in after the low power without touching the coverglass. Where they do not the barrel of the microscope must be raised before the high power is swung in. The high power should then be carefully lowered until it barely touches the coverglass and the drop brought into focus by adjusting upwards.

2. If difficulty is experienced in focusing on the drop under low power, focus first on the edge of the cover-glass. Then move the slide across and look for the drop.

3. An orange light is more suitable for observing hanging drops than white light or daylight.

4. Excessively long exposure to oxygen or an excessively rapid drop in tempe ture can result in an apparent nonmotili Motility tests should be performed quickly as possible after preparation of drop to avoid undue temperature chang In the case of organisms sensitive to oxyg the hanging drop test is not recommend Instead a small drop should be placed o slide, immediately covered with a cle coverslip, sealed on the margins with n lacquer and examined under oil immersi

Reference: Author's recommendation.

Leifson's Flagella Stain

Young liquid cultures are preferred this procedure.

Transfer the broth cultures to 15-r centrifuge tubes, and centrifuge at 30 r.p.m. for 5 minutes.

Pour off the supernatant and gently suspend the cells in about 7 ml. of distill water. Centrifuge. Pour off the supernata and gently resuspend the cells in distill water to give a just visible turbidity. I cubate at 37° C. for 10 minutes and che for motility.

Select a perfectly clean slide. Do not u a cloth or wash in soap or detergent.

Flame the slide for a few seconds a before it has cooled use a grease pencil mark a heavily defined band around area of about 1.5 square inches at one e of the flamed side of the slide. This acts a retarding margin around the stained ar

When cool, incline the slide, and pla loopfuls of the washed suspension at t points at one end of the enclosed area a allow them to run to the end. If this d not occur readily, the slide has not b correctly cleaned. Allow the slide to dry air and then place on a staining rack.

Shake the bottle of Leifson's stain th oughly. Using a pipette, slowly run 1.0 n of stain into the marked area with ca that the stain does not flow over the grea pencil marks.

Staining Time: Leifson states as follow "The proper staining time is best d termined by the formation of a fine p tate in the stain on the slide. As soon the precipitate is formed the flagella stained. The formation of the precipi- e is best observed by placing the slide on lack background and illuminating the in with a beam of light. The precipitate the appearance of rust coloured cloud ich generally starts forming along one e of the slide and then quickly spreads oughout the stain. As soon as the pre- itate is formed throughout the stain, time is up and the slide should be shed. This technique eliminates all guess rk as to the proper time to wash the les."

Flood the stain off the slide with a gentle eam of water. Counterstain for 1 minute h 1 per cent methylene blue.

(Note: It has been found in this labora- y that some flagella stain more readily er fixation in formalin.)

Preparation of the Stain: Three solutions prepared: (*a*) 1.5 per cent sodium chlo- le in distilled water, (*b*) 3.0 per cent nic acid in distilled water, and (*c*) 1.2 r cent basic fuchsin (special, for flagella in)* in ethyl alcohol. To effect complete lution, it is necessary to shake frequently r several hours.

Mix the three solutions in exactly equal portions and store either at −10° to 20° C. (stable indefinitely) or at 0° able for a few weeks) for 1 day or more clear before use.

Reproduced by permission from E. Leif- , J. Bacteriol., *62*, 1951, 377; and Stain chnol., *33*, 1958, 249; and from E. Leif- and L. Hugh, J. Bacteriol., *65*, 1953, 3.

liding Motility

The *Myxobacterales* and the *Beggiatoales* ide over the surface of solid media or ong solid surfaces. The larger forms are ry easily observed, but others, because their smallness and often very slow movement are observed with difficulty. To observe this movement remove the cultures from the incubator, and taking care to exclude air bubbles, cover a portion of the growth with a clean coverglass. Press it down gently to insure close contact, and examine with an oil immersion lens. Concentrate on small groups of cells at the margin of the colony and note their relative positions from time to time.

Preliminary observation of groups of cells separated at the margin of the main growth is usually indicative of gliding organisms. Their slime trails may be visible in the uncovered growth on the agar under the high dry power.

Gram Stain

Prepare a smear and fix by heat. Cover the smear with crystal violet and leave for 1 minute. Wash in a beaker for 3 or 4 seconds with tap water which should be running at a rate of about 30 ml. per second into a 250-ml. beaker. Rinse with Burke's iodine and leave the iodine on for 1 minute. Wash with water for a few seconds.

Hold the slide in a position where the smear is clearly visible against a white filter paper laid on the edge of the sink. Then apply 95 per cent alcohol, drop by drop, on the top edge of the smear until no more color runs out of the lower edge of it. The decolorization time is usually about 10 to 20 seconds. Wash; counterstain with .25 per cent safranin for 30 seconds. Wash, blot, dry, and examine.

Result: A blue color indicates Gram-positive; red, Gram-negative.

NOTE: The presence of a number of red cells among a mass of blue usually indicates the presence of dead, unstainable cells.

Mature spores are not stained by Gram and will appear as clear areas in the blue or red rod.

REAGENTS FOR THE GRAM STAIN

Crystal Violet

Crystal violet	2.0 gm.
Ammonium oxalate	1.0 gm.
Distilled water	100 ml.

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* Pharmaceutical Laboratories, National iline Division, 40 Rector Street, New rk.

Burke's Iodine

Iodine	1.0 gm.
Potassium iodide	2.0 gm.
Distilled water	100 ml.

Aqueous Safranin

Safranin 0, 2.5% solution in 95% ethanol	10 ml.
Distilled water	100 ml.

Reproduced by permission from J. W. Bartholomew, personal communication.

Cell Wall Stain

Procedure: Make smears of cultures on coverglasses, rather thickly to allow for loss of a portion of the material by washing.

Immerse the wet, unfixed smears in 1 per cent phosphomolybdic acid at room temperature (20° C. approximately) for 3 to 5 minutes. Stain with 1 per cent methyl green for 3 to 5 minutes. Wash well with water. Dry and examine under oil immersion. The cell walls stain dark green, and the cytoplasm is unstained.

Reproduced by permission from K. A. Bisset and C. M. F. Hale, Exptl. Cell Research, *5*, 1953, 449, Academic Press Inc.

Detection of Iron Deposited on Bacterial Cells or Their Capsules or Sheaths

Prepare a dense smear of the cells on a clean slide, and cover the smear with a drop of a 0.1 per cent aqueous solution of potassium ferrocyanide. Place a coverglass over the preparation and observe under the low or high dry power of a microscope.

Place a drop of N/3 sulfuric acid on one edge of the coverslip, and draw it under with a piece of filter paper placed on the opposite side. When the acid reaches the cells, the ferric iron dissolves and reacts with the potassium ferrocyanide to form Prussian blue.

Care must be taken to see that the cells are first carefully washed to remove soluble ferric iron which may still adhere from the medium.

Reference: Author's recommendat
based on material from an unknown sou

Neisser's Stain for Volutin or Me chromatic Granules

METHOD

1. Cover the fixed preparation w Neisser's blue for 2 minutes.
2. Wash the Neisser blue off the pre ration with chrysoidin, and allow the ch soidin to remain on the preparation fo to 5 minutes.
3. Wash lightly with running water.
4. Dry and examine.

Result: The metachromatic granu (volutin) stain bluish black whereas rest of the cell stains a light brown.

NOTE: If difficulty is experienced in tecting the cells repeat the stain but o the final washing with water. Remove excess chrysoidin with a filter paper dry.

SOLUTION A

Methylene blue	1
Alcohol, 95%	50
Glacial acetic acid	50
Distilled water	1000

SOLUTION B

Crystal violet	1
Alcohol, 95%	10
Distilled water	300

Before use mix together 2 parts of So tion A and 1 part of Solution B. This m ture is Neisser's blue.

CHRYSOIDIN

Chrysoidin	1
Distilled water	300

Dissolve by gentle heat and filter.

Reference: M. Neisser, Hyg. Rundsch *13*, 1903, 705. Reproduced by permiss from Lange and Springer, Berlin.

Detection of Calcium Carbonate Cells

1. *Evolution of Carbon Dioxide:* Mo dense suspension of the cells on a

er a coverslip. Examine under the low high power of the microscope. Draw HCl under the coverslip by means of a e of filter paper applied to the opposite . Note any evolution of gas from the s.

. *Staining for Calcium:* Fix the cells in per cent formalin. Centrifuge and sus- d the packed cells in a small quantity .1 per cent alizarin red S for 1 hour. sh and counterstain in 0.1 per cent eous toluidine blue, thionin, or meth- ne blue.

Calcium deposits appear red.

Reproduced with modification by per- sion from Medical Public Health Lab- tory Methods, p. 1081, Lea and Febiger, iladelphia, 1955.

cchiavello's Stain for Rickettsiae

tain a fixed smear with fuchsin solution filtering it over the preparation through oarse filter paper in a funnel) for 4 min- s. Drain and wash rapidly with 0.5 per t citric acid solution.

Wash immediately with tap water. Stain h a 1 per cent aqueous solution of meth- ne blue for 20 seconds.

The rickettsiae, intra- and extracellular stained red; the cellular elements, blue. is method is effective for culture smears not for tissue sections of typhus-in- ed animals.

uchsin Solution: 0.25 per cent solution basic fuchsin in a M/30 phosphate solu- n buffered to pH 7.4.

Reproduced by permission from D. T. ith and N. F. Conant, Zinsser's Text- k of Bacteriology, 11th ed., Appleton- ntury-Crofts, New York, 1957.

iemsa Stain for Chromatinic Struc- res in Bacterial Cells

Make an impression preparation from a y young agar culture, and expose it to vapor of a freshly prepared 2 per cent nic acid solution for 2 to 3 minutes.

Place the slide in N HCl at 60° C. for 10 utes to hydrolyze the cytoplasmic proteins and render them less stainable. Wash thoroughly with distilled water.

Stain with Giemsa for 30 to 60 minutes. Wash thoroughly and mount in water.

The chromatinic structures stain a darker color than the rest of the cells.

Reproduced by permission from C. F. Robinow, in Addendum to R. J. Dubos, The Bacterial Cell, Harvard University Press, Cambridge, 1949.

A Method for Staining the Nuclei of Blue-Green *Algae*

Place filaments of the alga on a clean slide.

Flood with Bouin's fluid for 4 to 6 hours.

Carefully wash off the Bouin's fluid with tap water until none of it remains.

Flood with 1:20 dilution of Giemsa for about 40 hours.

Wash with tap water.

Allow to dry thoroughly before mounting in Depex mounting medium.

The nuclei stain a dark blue against a paler background.

Reference: Author's recommendation.

Toluidine Blue Stain for Nuclear Structures in Yeast Cells

Make a smear of yeast cells from a 6- to 8-hour culture, and allow it to dry in air. Flood the slide with 40 per cent ethanol for 2 minutes. Wash with tap water.

Flood with 0.1 M KOH for 1 hour at 28° C. Wash well with tap water.

Stain with 0.1 per cent toluidine blue in 10 per cent ethanol for 2 minutes. Wash with 10 per cent ethanol. Blot the slide and dry before mounting.

The nuclei stain a dense blue against a pale pink background.

Reproduced by permission from G. F. Townsend, Stain Technol., *32*, 1957, 302.

A Method of Staining the Nuclei of *Fungi*

Place sterile slides in a humid chamber and drop onto each, 1 drop of a liquid Czapek-Dox or other suitable medium.

Inoculate the drop with fungal spores and then incubate at 28° C. for 18 hours.

Carefully flood the slide with Bouin's fluid for 4 to 6 hours in the moist slide chamber. Remove the slides and wash carefully with tap water until all the Bouin's fluid is removed.

Stain with 1:20 dilution of Giemsa for 24 to 48 hours.

Wash with water and allow to dry before mounting in Depex mounting medium.

The nuclei stain a dark blue against a paler background.

Reference: Author's recommendation.

Heidenhain's Iron Hematoxylin Method

Fix the preparation in alcohol or by exposure to the vapor of 2 per cent osmic acid for 1 minute.

Mordant with 5 per cent iron alum for 10 to 20 minutes.

Rinse rapidly in distilled water. Stain with Heidenhain's iron hematoxylin solution for 10 to 20 minutes.

Differentiate in 0.5 per cent acid alcohol; control the degree by observation under the microscope. Rinse in tap water to arrest differentiation. Rinse finally in distilled water and blue with Scott's solution. Wash in running water for 5 to 10 minutes. Counterstain as desired. Dry and examine.

SOLUTIONS

1. 5 Per Cent Iron Alum in Distilled Water

Use the violet crystals of iron alum.

2. Hematoxylin Solution

Hematoxylin	5 gm.
Absolute alcohol	100 ml.

For use dilute 1:5 with distilled water.

3. Scott's Bluing Solution

$NaHCO_3$	3.5 gm.
$MgSO_4 \cdot 7\ H_2O$	20.0 gm.
Distilled water	1000 ml.

Dissolve the salts separately, mix, and add a crystal of thymol to preserve.

Bouin's Fluid (Fixative)

Picric acid, saturated aqueous solution	75 m
Formalin	25 m
Glacial acetic acid	5 m

Giemsa Stain

Azur II-eosin	3 gm
Azur II	0.8 gm
Glycerol (pure)	250 gm
Methanol (acetone-free)	250 gm

Giemsa Stain (Alternative)

With a pestle and mortar grind 0.5 gm of Giemsa powder with 33 ml. of glycero at 55° to 60° C. for 1.5 to 2 hours. Add 3 ml. of methanol and allow to stand for 2 hours.

The Ziehl-Neelsen Stain for Acid-Fas Bacilli

1. Place the fixed preparation on the ho plate.

2. Flood the slide with concentrate carbolfuchsin. Continue to heat the slid for a period of 10 minutes, and add fres stain to it if there is any tendency for th original stain to dry off.

3. Wash the slide thoroughly with ta water, and then, with a filter paper mois tened with alcohol, carefully clean all th stain deposits from the back of the slid and also from the surface of the slide awa from the smear itself. Wash again wit water.

4. Immerse the slide in 25 per cent sul furic acid for 5 minutes. The red stain im mediately turns brown and begins to de- colorize. Remove the slide and rinse unde running water. As the acid is washed ou any remaining stain deposit will again tak on its original pink color. Continue th sulfuric acid treatment until the smea upon washing with water, is colorless o only a very faint pink. Finally, wash we with water. Never prolong treatment beyond 20 minutes.

5. Counterstain with Loeffler's meth- ylene blue for 2 minutes.

Wash lightly with running water, dry, examine. Acid-fast organisms usually ar as isolated bundles of 3 to 6 organ- in tuberculous sputa and stain red in ie background.

)TE: The prolonged treatment in 4 above ecessary only for the removal of stain ı extraneous material. Nonacid fast nisms decolorize in a few seconds.

FFLER'S METHYLENE BLUE

hylene blue, saturated solution alcohol	30 ml.
assium hydroxide, 0.0001% queous	100 ml.

CENTRATED CARBOLFUCHSIN

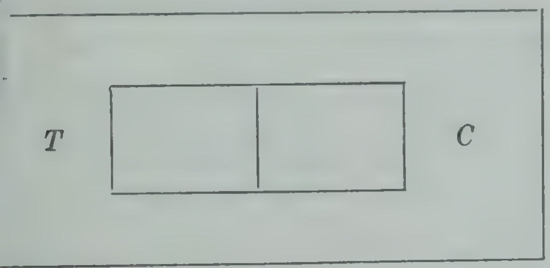
ic fuchsin	1.0 gm.
olute alcohol	10 ml.
nol, 5% aqueous	100 ml.

)issolve the dye in the alcohol and add phenol solution.

le Agglutination Test

This test is usually employed as a qual- ive test for the detection of antibodies a particulate antigen. It should be ap- ent, however, that, with the exercise of ittle care, the technique could be used quantitative purposes.

'repare two wax cells on a slide, as illus- ed below, by dipping the end of a hot e in wax and pressing it down on the face of a second slide.



Place a drop of 0.85 per cent NaCl in mpartment *C* and with the same pipette ce a drop of antiserum (1:20 for "O" or 100 for "H") in compartment *T*.

With another pipette, transfer one drop antigen (approximately 3×10^9 cells ml.) to both *T* and *C*.

With a wire loop, mix the contents of *C* and then of *T*. Sterilize the loop, and then rock the slide with a fairly rapid rolling motion, holding it in such a position over a lamp so that the cells can be clearly observed. Clumping of the cells in *T* without similar clumping in *C* is a positive agglutination test. Clumping in *C* indicates an unstable auto-agglutinable suspension which is unsuitable for serological work.

Reference: Author's recommendation.

A Technique for Dispensing Oxygen-Free Media and for Subculturing of Strictly Anaerobic Organisms

An important and often overlooked factor in the successful culture of anaerobes is the virtual total exclusion of oxygen during the collection and subsequent handling of the samples. The most explicit description of a technique which has proved successful in the hands of several investigators is that of Hungate described below. Such precautions are not necessary with all anaerobes but should be adopted if less exacting methods fail.

Method

Prepare oxygen-free CO_2, H_2, or N_2 by bubbling the cylinder gases with low O_2 content through a chromous acid solution prepared as follows.

To 75 gm. of twenty 20 mesh metallic zinc add 50 ml. of 3 N HCl and stir vigorously for 30 seconds. Then dilute 2.5 ml. of a saturated aqueous solution of mercuric chloride to 50 ml.; add this and stir the mixture for 3 minutes after the evolution of gas ceases.

Wash the zinc by decantation, and place it in the gas-washing bottle. Then dissolve 50 gm. of chromium potassium sulfate, $Cr \cdot K \cdot (SO_4)_2 \cdot 12\ H_2O$, in a minimal amount of distilled water, and place the solution in the washing bottle; also add 10 ml. of 5 N H_2SO_4.

Pass the cylinder gas through the bottle to displace all air and allow to stand for 24 hours. The slow evolution of H_2 from the

Zn reduces the chromium, and the solution turns a brilliant clear blue. The reduction of the chromium can be speeded up by forcing the solution back and forth through the zinc several times.

This preparation effectively absorbs oxygen for weeks, even months, if the gas contains only small quantities. Tank nitrogen contains more oxygen than the other gases, and the absorbent is soon exhausted. In such a case use hot reduced copper instead of, or preceding, the chromous solution. Alkaline pyrogallol removes not only oxygen but also carbon dioxide which may be required for the growth of some organisms and which stimulates many others. Bicarbonate-CO_2 buffers cannot be employed when pyrogallol is the absorbent. It is therefore not recommended.

TECHNIQUE FOR DISPENSING AN ANAEROBIC CULTURE MEDIUM WITH MINIMAL ACCESS OF AIR

1. For Media Which Cannot Be Heat-Sterilized: In applying this technique, it is presumed that the medium to be transferred has been prepared and sterilized under conditions in which oxygen has been rigidly excluded. The technique as described is intended for the preparation of roll tubes. Modification for other purposes should be obvious.

Sterilize empty culture tubes (16- by 150-mm. Pyrex tubes without lips) without cotton wool plugs and upon removal from the autoclave close with sterile rubber stoppers.

Transfer the medium as follows. Connect two bent Pasteur pipettes with the gas source via a Y-tube and insert one in the culture tube and one in the flask of medium; flush out all the air.

Insert a sterile cotton-plugged 10-ml. pipette provided with a mouth suction tube into the gas above the culture medium in the flask and suck gently so that air in the pipette is replaced with CO_2. The taste indicates when this has been accomplished.

Then draw in the medium and transfer 10 ml. quickly to the culture tube.
the last drop, but do not blow throu
pipette. Stopper the tube and keep
C. until it has been inoculated. To s
the tube, hold it between the palm a
last two fingers of one hand, and h
stopper between the index finger and
of the same hand. Insert the stopper
capillary is withdrawn. Then finally
both hands and a rotary motion se
stopper. The stopper can be seated
tightly by tipping so that the mediu
the lower surface of the stopper; th
of agar which occupies the space b
the stopper and the glass then effe
prevents the entrance of air.

Culture tubes closed in this way
been found to remain anaerobic alm
definitely. The *absolute* exclusion of
by means of a rubber stopper is not c
or implied, but any oxygen that is p
is not sufficient to change the color
oxidation-reduction indicator.

2. For Media Which Can Be Ste
by Heat: Dispense the medium in
5-ml. quantities in 150- by 16-mm.
Flush with a mixture of 80 per cent
gen and 20 per cent carbon dioxid
seal with rubber stoppers. Secure the
pered tubes in a rack so designed th
stoppers cannot be blown out of the
during sterilization; then sterilize at 1
for 20 minutes.

ANAEROBIC PROCEDURE FOR INOCUL AND DILUTION

1. When Tubes Must Be Opened
Transfer: To inoculate, extend the
end of a bent sterile capillary pipett
6 cm. into the gas space above the m
in the first dilution tube. Stand the st
upside down on a clean surface and
until it is to be reinserted. Insert a s
bent sterile pipette into the tube
which a colony is to be picked. Pass
gas through both tubes during the
time the stoppers are removed.

Flame a Pasteur pipette; cool i
medium to be inoculated and use t

p the colony which is to be trans- to the first dilution tube. Stopper st dilution tube with minimal access and mix thoroughly. Then open and ain with the same capillary.

a sterile 1-ml. pipette with a large vith the oxygen-free gas in the tube ansfer 0.1 or 0.2 ml. of this dilution e 2. Close tube 1 aseptically with the ion of air. Flame the capillary and r tube 3 and so on. While doing this, he tubes immersed to half their depth ater bath at 45° C.

l the inoculated tubes under running , rolling them in the horizontal position o keep the medium flowing over the surface of the tube until just before gar is ready to set.

en invert the tubes momentarily for st time, thus forming a seal of medium d the stopper and leaving on the portion of the tube a thin film of which will solidify almost immedi.

ld the tubes stationary in a vertical on under the tap until the rest of the has set.

When the Tubes Are Not Opened during sfer (as in Preparations of Liquid Dis): Make transfers with a sterile hymic syringe and a 1 3/4 inch. 19-gauge e. Flush the syringe several times the sterile gas mixture, and then in-hrough the sterilized rubber stopper; fer the required sample to the next mix, and repeat the process until ons are complete.

TE: Total exclusion of oxygen may impossible or, as Hungate suggests, in its total absence, the E_h of the ium may be too high for some anbes. To control the entry of minor nts of oxygen and to establish a low Hungate has employed the following od.

the medium during preparation, add per cent sodium dithionate and 0.1 ent sodium pyruvate. Dispense and ze. Inoculate with 2 per cent inoculum of a broth culture of *Escherichia coli* and incubate for 12 hours at 39° C. Then heat the cultures to 75° for 45 minutes to kill *E. coli*; cool and inoculate.

For many organisms 0.1 per cent cysteine may be employed in place of the dithionate should the latter be too toxic. Cysteine will not, however, develop the low potential obtainable with dithionate or by the *E. coli* oxidation of pyruvate.

Solid media may be prepared by addition of agar to the liquid media. The techniques are the same.

Reproduced by permission from R. E. Hungate, Bacteriol. Revs., *14*, 1950, 1; and from P. H. Smith and R. E. Hungate, J. Bacteriol., *75*, 1958, 713.

Transfer in an Oxygen-Free Atmosphere

Consideration may now be given to the use of collapsible plastic bags for anaerobic transfers. One of the major difficulties in the use of anaerobic chambers has been that of removing the air which necessitates evacuation and repeated flushing with an oxygen-free gas. The construction of a transfer chamber, consisting of a large polythene bag fixed to a ring on a flat metal plate containing two 15-cm. armholes sealed with arm-length surgical gloves, an entry port, and a gas inlet tube, offers some advantages over the method of Hungate.

The bag may be inflated with any inert oxygen-free gas; the cultures, culture media, and sterile transfer instruments are inserted through the port in an outflow of the gas, and the bag is reinflated once or several times before transfer is effected.

Reference: Author's recommendation.

Pyrogallic Acid-Sodium Carbonate Plates for the Culture of Anaerobes

Two grams of pyrogallic acid mixed with an excess of sodium hydroxide will absorb at least 200 ml. of oxygen. When sodium carbonate is used, carbon dioxide is liberated in the formation of the sodium pyrogallate. This materially aids the growth of most anaerobes.

To prepare single anaerobic plates, inoculate the medium and then invert the base over a piece of sheet glass or the unused lid of a 2-lb. can (obtainable from most can manufacturers). Fold a filter paper double, and place in the center of the fold approximately 1 gm. of pyrogallic acid and 1 gm. of washing soda. Slip the fold between the edge of the petri dish and the glass plate and pull firmly outwards to compress the sealed reagents agains side of the dish. Take care that it doe touch the agar. If so, reduce the qua of reagents. Tear off the protruding and seal with a mixture of 50 per petrolatum and 50 per cent paraffin The wax should be heated until vapors rise from it before being used it should be possible to ring the dish wax from a pipette before solidific takes place. Wax which is too coo crack and later admit air.

Reference: Author's recommendatio

A GUIDE TO STUDY

e following is a suggested grouping of rial genera for purposes of study. e important common characters and estions as to the type of differentiating acters which should be sought, tabu-, and memorized, are given.

xcept where specifically stated other- the organisms should be regarded as ellular, rigid, and straight.

ed and Purple Photosynthetic ur Bacteria

ll contain bacteriochlorophyll and car- oid pigments with the latter predom- ing to give a reddish-purple color.

ll deposit sulfur internally when ex- d to H_2S.

ll are anaerobic so far as is known.

ifferentiation is based on the shape and ngement of cells.

osarcina, *Thiopedia*, *Thiocapsa*, *Thio- yon*, *Thiothece*, *Thiocystis*, *Lamprocystis*, *oebobacter*, *Thiopolycoccus*, *Thiospiril-*, *Rhabdomonas*, *Rhodothece*, *Chroma-*.

ed and Purple Photosynthetic sulfur Bacteria

li grow anaerobically with exposure to t and obtain energy both from oxidation arbon compounds and from radiation. ne species of *Rhodopseudomonas* and sibly *Vannielia* will grow aerobically the dark. Differentiation is based on rphology.

odopseudomonas, *Rhodospirillum*, *Rhodo- robium*,* *Vanniela*.

Green Photosynthetic Sulfur Bac- ia

ll contain chlorobium chlorophyll so as is known.

ll are anaerobes.

All deposit sulfur externally in the presence of H_2S so far as is known.

Differentiation is based on morphology and association with symbionts.

Chlorobium, *Pelodictyon*, *Clathrochloris*, *Chlorobacterium*, *Chlorochromatium*, *Cylindrogloea*.

4. Nonphotosynthetic, Strictly Autotrophic, Straight, Gram-negative Rods with Polar Flagella if Motile

All fail to grow on organic media.

Differentiation is based mainly on morphology and on nutritional differences.

Nitrosomonas, *Nitrosocystis*, *Nitrosogloea*, *Nitrobacter*, *Nitrocystis*, *Thiobacillus*,* *Ferrobacillus*.

Note: Other autotrophs *not* included above are *Nitrosococcus* and *Nitrosospira*, neither of which are straight *rods*.

5. Nonphotosynthetic, Facultatively Autotrophic, Straight, Gram-negative Rods with Polar Flagella

These organisms are heterotrophic and grow well on organic media but may need a special gas phase. They can also be grown autotrophically with energy obtained from the oxidation of simple inorganic substances.

Differentiation is based on the energy substrate.

Thiobacillus,† *Methanomonas*, *Hydrogenomonas*, *Carboxydomonas*.

6. Miscellaneous Colorless, Nonphotosynthetic Organisms Depositing Sulfur Internally

Differentiation is based principally on morphology.

Also included in another group.

* Also has some facultatively heterotrophic species.

† Some species only.

Thiovulum, Macromonas, Thiospira, Thiobacterium.

7. Nonphotosynthetic, Heterotrophic, Gram-negative Rods with Polar Flagella

Differentiation is based on morphology, pigmentation, luminescence, nitrogen fixation, salt tolerance, and biochemical reactions, particularly in regard to the type of compounds oxidized and the byproducts produced.

Pseudomonas, Xanthomonas, Acetomonas (*Acetobacter*), *Aeromonas, Photobacterium, Azotomonas, Zymomonas, Protaminobacter, Alginomonas, Mycoplana, Zoogloea, Halobacterium.*

8. Colorless, Nonphotosynthetic Organisms Normally Fixed to a Substratum by Means of a Stalk

They do *not* form sheaths.

Differentiation is based on morphology, the nature of the stalk, deposition of iron, and mode of division.

Caulobacter, Gallionella, Siderophacus, Nevskia, Pasteuria, Blastocaulis.

9. Colorless Nonphotosynthetic Organisms Noted for Deposition of Iron in Capsules or on Cells

Do not form sheaths or stalks.

Differentiation is based on cell morphology, presence or absence of capsules, and mode of iron deposition.

Siderocapsa, Siderosphaera, Sideronema, Ferribacterium, Sideromonas, Naumanniella, Ochrobium, Siderococcus, Siderobacter, Ferrobacillus, Thiobacillus.**

10. Colorless, Nonphotosynthetic Organisms with Distinctly Curved Axis; No Stalk Formation; Motile with Polar or Lateral Flagella

Differentiation is based on morphology, location of flagella, mode of reproduction, specific metabolic characters, and oxygen tolerance.

Vibrio, Desulfovibrio, Methanobacterium, Cellvibrio, Cellfalcicula, Microcyclus, M plana, Selenomonas, Photobacteri Thiospira.**

11. Organisms with Helical (Spi Axis; Motile with Polar Flagella Nonmotile

Differentiation is based on photo thetic pigments, and on morphological nutritional characters.

Rhodospirillum,† Spirillum, Paraspiril Myconostoc, Nitrosospira.

12. Organisms Occurring in Sheat

Differentiation is based on unicellula multicellular forms, morphology, intra lular deposition of sulfur, deposition iron, type of motility (if any), presenc aerosomes, and type of sheath.

Sphaerotilus, Leptothrix, Toxothrix, ploca,‡ Pelonema, Crenothrix, Phragmi thrix, · Clonothrix, Thioploca,* Thiothr Coleomitus* (?).

13. Organisms Reproducing by truding a Tube from the End of Cell

Differentiation is based on pigmenta and oxygen sensitivity.

Rhodomicrobium,§ Hyphomicrobium.

14. Colorless Organisms Capable Fixing Atmospheric Nitrogen N symbiotically

Differentiation is based on morpholo type of flagellation.

*Azotobacter, Azotomonas,¶ Azotococ Beijerinckia, Aerobacter.**

15. Gram-negative Rods with P trichous Flagella which Prod Water-insoluble Pigments

Differentiation is based principally the nature of pigment but also on chemical reactions.

* Also in Group 4.

* Also listed in other groups.
† Included in another group.
‡ Also included in another group.
§ Also included in a previous group.
¶ Also included in group 7.

nobacterium, Flavobacterium, Serratia, richia.*

Colorless, Gram-negative Rods lucing Nodules and Malformations Roots of Plants

fferentiation is based on the nature of nodule or malformation and the fixa- of nitrogen.

obium, Agrobacterium.

Colorless, Gram-negative Rods, Peritrichous Flagella or Non- ile, Growing Well on Meat Infu- Agar Aerobically

ifferentiation is based on hydrolysis of , alginates, chitin, pectins, and gelatin; ence or absence of flagella; production cid or acid and gas in glucose; fermen- on of lactose (positive or negative and d or delayed); production of indole and ise; methyl red and Voges-Proskauer tions; pathogenicity; and temperature rowth.

aligenes, Achromobacter, Agarbacterium, eckea, Escherichia, Aerobacter, Klebsi- , Paracolobactrum, Alginobacter, Er- ia, Proteus, Salmonella, Shigella, Herel- Mima, Colloides.

Small, Colorless, Unicellular Rods Nonenteric Origin Requiring ino-Nitrogen and Frequently wth Factors

erobic, Gram-negative.

ifferentiation is based on nutritional irements, liquefaction of inspissated m, motility, and pathogenicity.

teurella, Bordetella, Brucella, Haemo- lus, Actinobacillus, Calymmatobacterium, raxella, Noguchia, Streptobacillus.

Anaerobic, Gram-negative Rods

Differentiation is based on morphology e, shape, and pleomorphism), motility, l nutritional characters and reaction in tone water.

teroides, Fusobacterium, Dialister, aerophorus.

20. Gram-positive Cocci

Differentiation is based on morphology, arrangement of cells, sensitivity to oxygen, production of gas from peptone, production of methane, bile solubility, catalase, production and rotation of lactic acid, and utilization of ammoniacal nitrogen.

Micrococcus, Staphylococcus, Aerococcus, Gaffkya, Sarcina, Peptococcus, Diplococcus, Streptococcus, Pediococcus, Leuconostoc, Peptostreptococcus Nitrosococcus.

21. Gram-negative Cocci

Differentiation is based on morphology and arrangement and on sensitivity to oxygen.

Mima, Herellea, Colloides, Lampropedia, Neisseria, Veillonella.

22. Aerobic, Gram-positive, Nonsporing, Nonacid-fast, Unbranched Rods*

Differentiation is based on morphology and arrangement of cells, motility, production of acid or acid and gas from glucose, production of acid from lactose, fermentation of lactate, acidification and clotting of milk, tolerance of high temperatures, tolerance of low pH, hydrolysis of cellulose, cyclic development as cocci and rods, and pathogenicity.

Lactobacillus, Propionibacterium, Corynebacterium, Listeria, Erysipelothrix, Microbacterium, Cellulomonas, Kurthia, Brevibacterium, Arthrobacter, Leptotrichia.

23. Anaerobic, Gram-positive, Nonsporing, Nonacid-fast, Unbranched Rods

Differentiation is based on morphology and arrangement of cells, motility, production of butyric and propionic acids from lactate, production of gas from peptone, and production of large amounts of alcohol from glucose.

Butyribacterium, Propionibacterium,† *Ca-*

Some strains only.

* Odd bifurcations are not considered here to be branches.

† Included also under aerobic rods.

*tenabacterium, Ramibacterium, Eubacterium, Cillobacterium, Leptotrichia**.

24. Rod-shaped, Unicellular Organisms Producing Endospores

Differentiation is based on sensitivity to oxygen and production of methane.
Bacillus, Clostridium, Methanobacterium.

25. Gram-positive, Acid-fast Nonmotile Multicellular Rods

The only genus represented is *Mycobacterium.*

26. Branching Gram-positive Rods

Differentiation is based on formation of a true branching mycelium in the early stages of growth, persistence of the branching character, multicellularity, production and arrangement of conidia, production and the nature of sporangia, motility of conidia, sensitivity to oxygen, and optimal temperature of growth.
*Arthrobacter,** *Mycobacterium,** *Propionibacterium,** *Nocardia, Jensenia, Actinomyces, Thermoactinomyces, Micromonospora, Waksmania, Streptomyces, Actinoplanes, Streptosporangium.*

27. Organisms Clearly Multicellular without Application of Special Cell Wall Stains

Rigid or flexible; some cells greatly exceed 2 μ in diameter.

Differentiation is based on morphology, presence or absence of a sheath, motility, endospores, flagella, intracellular deposition of sulfur, flexibility of cells, and sensitivity to oxygen.
Caryophanon, Simonsiella,† *Oscillospira, Arthromitus, Coleomitus, Beggiatoa, Thiospirillopsis, Thioploca, Thiothrix, Leucothrix, Vitreoscilla.*

28. Aerobic, Gram-negative Ro Forming Long, Motile, Articulat Chains

Differentiation is based on the presen or absence of flagella.
Lineola, Bactoscilla.

29. Flexible, Rod-shaped Organism Motile with Gliding Action on Sol Surfaces or along Adjacent Cells i Liquids

No flagella; unicellular; Gram-negativ

Differentiation is based on the size of t cells, presence or absence of microcyst and the development and nature of fruiti bodies.
Microscilla, Cytophaga, Sporocytophag Archangium, Stelangium, Sorangium, Pol angium, Synangium, Podangium, Chondr myces, Myxococcus, Chondrococcu Angiococcus.

30. Colorless, Flexible, Spiral-shape Organisms without Flagella, Activel Motile in Solutions

Differentiation is based on the size of t cells, presence of an axial filament or crista which is visible with a standa microscope, sensitivity to oxygen, hooki of cell ends, and general morphology.
Spirochaeta, Saprospira, Cristispira, Bo relia, Treponema, Leptospira.

31. Pleuropneumonia and Pleur pneumonia-like organisms

Mycoplasma

32. Intracellular Parasites of Man ar Animals, Arthropods and Trematod

Differentiation is based on the type tissue or blood cells invaded, nature growth in or on the cells, growth in chick embryos, morphology, nature of the di ease produced, symptoms of the diseas transmission by arthropods or trematod and mode of transmission by arthropods
Rickettsia, Coxiella, Ehrlichia, Cowdri Neorickettsia, Wolbachia, Symbiotes, Ricke siella, Chlamydia, Colesiota, Ricoles Colettsia, Miyagawanella, Bartonella, G hamella, Haemobartonella, Eperythrozo Anaplasma.

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* These genera have been included in other groups. They do not branch in the accepted sense but may, under some conditions of growth, produce bifurcated cells or angulated cells.

† It is doubtful whether this is multicellular. It resembles a large, nonmotile *Streptococcus.*

INDEX

he following Index the generic name and the page upon which the corresponding generic
appears are printed in boldface.

te, 6, 8, 13, 23, 25–27, 42, 55, 56, 62, 63,), 71, 96–98, 103, 106–108, 110, 111, 117
  ntification of, 174
bacter, 27, 62–64, 186, 208, Plate 7, ig. 2
monas, 27, 62
yl-methyl-carbinol, 109
omatium, 6, **131,** Plate 27, Fig. 3
omobacter, 32, 33, 36, 38, 39, 41, 86, **87,** 3. 209
fast, 21, 23, 118, 120, 121, 202, 210
obacillus, 33, 35, 37, **76,** 209
omyces, 20, 23, 77, **120,** 210, Plate 22, Fig. 5
oplanes, 20, 21, 122, **127,** 210, Plate 23, Fig. 1
osine triphosphate, 34, 37, 76
bacter, 27, 30, 31, **90,** 91, 92, 93, 208, Plate 14, Fig. 3
coccus, 14, 99, **100,** 101, 209
monas, 34, 38, **63,** 64, Plate 7, Fig. 3
somes. *See* Gas vacuole
, clarified, 196
drolysis of, 28, 30, 36, 39, 40, 61, 66, 70, 181
rbacterium, 30, 36, 39, 40, **88,** 89, Plate 13, Fig. 6
bacterium, 28, 32, 39, 41, **85,** 209, Plate 13, Fig. 3
ligenes, 36, 38, 40, 41, 85, **86,** 87, 209, Plate 13, Fig. 5
hol, 41, 48, 63, 70, 109, 116, 171, 209
  etermination of, 175
  ast-water, 27, 186
e, 1, 5, 9, 17, 44, 69, 73, 81
nate agar, 182
nic acid, 28, 31, 41, 65, 88, 93, 120, 182, 209
nobacter, 31, **93**
nomonas, 28, 40, 41, **65,** 66, 208, Plate 8, Fig. 1
nentary tract, 12, 41, 70, 87, 90, 97, 98, 102, 105, 107, 110, 111, 128
aloids, 171
ylamine, 28, 36, 38, 65
edium for *Protaminobacter*, 146, 156
oebobacter, 48, **50,** 51, 207, Plate 2, Fig. 4
ines, 25, 108, 109
monia, 6, 9, 13, 19, 25, 56–58, 60, 62, 75, 83, 86, 88–90, 100, 102, 104, 111, 114–116, 118, 120, 122, 132, 136
  uantitative determination of, 148
abaena, 130
aerobic, 6, 7, 8, 9, 11, 12, 13, 17, 19, 21, 23–26, 42, 52, 54, 55, 59, 65, 70, 71, 73, 90, 96–98, 102, 103, 106–111, 115, 116, 120, 121, 126, 137, 207, 209
  methods, 203–206

Anaplasma, 46, **141,** 142, 210
Andrade's indicator, 172
Angiococcus, 47, 135
Aphanothece, 49, Plate 1, Fig. 5
Archangium, 47, **132,** 133, 210, Plate 27, Fig. 5
Arizona group, 30, 92
Arthrobacter, 12, 22, 29, 33, 35, 36, 40, **114,** 115, 119, 120, 209, 210, Plate 21, Figs. 1–5
Arthromitus, 2, 16, **128,** 210, Plate 25, Fig. 4
Arthropods, 45, 46, 138, 141, 210
Arthrospores, 27, 84
Ascitic fluid, 34, 35, 48, 70, 78, 96, 98
Autotrophs, 9, 13, 17, 19, 29, 38, 41, 54–59, 70, 83, 101, 129, 207
Axial filament, 4, 10, 136, 137
Azotobacter, 6, 7, 27, **84,** 85, 208, Plate 12, Fig. 5
Azotococcus, 27, **85,** 208
Azotomonas, 27, 31, **64,** 208, Plate 7, Fig. 5

Baar's medium for *Desulphovibrio*, 193
Bacillus, 19, 27, **115,** 116, 210, Plate 22, Fig. 1
Bacterial cell suspension agar, 18, 135, 183
Bacteriochlorophyll, 4, 5, 9, 14, 17, 18, 48–55, 158, 207
Bacterium, 22, 28–33, 39
  albilineans, 28
  alkaligenes, 38, 41
  anitratum, 12
  healii, 23
  nenckii, 30
  parapelomyxae, 30, 32
  pneumosintes, 42
  stewartii, 28, 34
  tardicrescens, 28, 32, 39
Bacteroides, 42, 43, **96,** 209
Bactoscilla, 16, 19, **130,** 210, Plate 26, Fig. 2
Ballerup group, 31, 32, 39, 92
Bartonella, 12, 40, 46, **140,** 141, 210
Beggiatoa, 3, 16, 81, 82, **129,** 210, Plate 25, Fig. 6
Beijerinckia, 27, 85, 146, 208
Beneckea, 39, **89,** 209, Plate 14, Fig. 1
Benzidine reagent, 164
Bethesda group, 31, 32, 39, 92
Bicellular, 85
Bifidobacterium, 26
Bifurcation, 24, 26, 42, 107, 209
Bile, 13, 14, 99, 104, 105, 209
  solubility test, 194
Bipolar staining, 12, 74
Blastocaulis, 4, 11, **84,** 208, Plate 12, Fig. 6
Blood, 14, 27, 31, 33–35, 40, 42, 70, 73, 76, 78, 97, 100, 102, 103, 105, 107, 113, 116, 123
  agar, 187
  cystine-glucose, 188

Blue-green alga, 1, 2, 4, 49, 82, 84, 129, 130, 201
Bordetella, 37, 40, **74,** 75, 209, Plate 15, Fig. 6
Bordet-Gengou medium, 37, 74, 188
Borrelia, 11, **137,** 209, 210, Plate 30, Fig. 2
Bouin's fluid, 202
Branching, 4, 7, 8, 15, 20–26, 29, 30, 33–35, 64, 66, 80, 98, 107, 109–111, 114, 118, 120–123, 126–128, 138, 141, 210
Brevibacterium, 22, 23, **103,** 112, 209, Plate 18, Fig. 2
Brucella, 34, 37, **75,** 209, Plate 16, Fig. 1
Budding cells, 4, 8, 11, 16, 84
Butyl alcohol, 42, 55, 71, 102, 117, 118
2,3-Butylene glycol, 30, 34
n-Butyrate, 71, 96, 98, 101
Butyribacterium, 25, 26, 108, 109, **111,** 210, Plate 19, Fig. 6
Butyric acid, 24, 25, 26, 42, 55, 107, 108, 111, 112, 209
  identification of, 174

Calcium carbonate, 6, 52, 59, 131
  detection of, 200
Calymmatobacterium, 26, **77,** 188, 209, Plate 16, Fig. 4
Capsule, 2, 5, 9, 11, 13, 14, 17–19, 29, 32–34, 43–45, 49, 50, 52, 53, 57, 63, 64, 67, 68, 72, 73, 77–79, 87, 91, 92, 101, 104, 105, 115, 200, 208
Capsular polysaccharide medium for *Saccharobacterium,* 183
Carbon dioxide, 6, 7, 19, 23–25, 27, 36, 37, 40, 41, 42, 56, 58, 60, 65, 70, 71, 75, 101, 102, 106–108, 110, 111, 116, 153, 172
  determination of, 176
  preparation of, 153
Carbon monoxide, 19, 59, 101, 151, 152, 176
  determination of, 176
  preparation of, 152
Carboxydomonas, 19, **58,** 59, 147, 150, 152, 207
  autotrophic medium for, 147, 150
Carotenoid, 4, 5, 9, 11, 48, 50, 51, 54, 55, 58, 67, 158, 207
Carrot plug medium, 184
Caryococcus, 13
Caryophanon, 2, 6, 15, **127,** 210, Plate 23, Fig. 6
Catalase, 22, 23, 26, 65, 75, 79, 90, 99, 100, 102, 105, 108, 110, 113, 115, 209
  test for, 195
Catenabacterium, 24, 25, 26, 108, **109,** 210
Caulobacter, 17, **82,** 209, Plate 8, Fig. 5
Cell, 1
  walls, 1, 17
  stain for, 200
Celfalcicula, 29, **72,** 157, 208, Plate 10, Fig. 5
Cellulomonas, 22, 29, 33, 36, 41, **114,** 209, Plate 20, Fig. 6
Cellulose, 22, 24, 29, 61, 63, 70, 114, 117, 123, 126, 132, 135, 136, 156, 209
  mineral salts medium, 71, 72, 147
  oxidation to oxycellulose, 29, 72, 157
  precipitated, 114, 132, 156
  peptone medium, 156
Cellvibrio, 29, **71,** 157, 208, Plate 10,
Chain, 1, 2, 3, 5, 6, 8, 9, 13, 14, 15, 17,
24, 26, 27, 42, 48, 68, 69, 71, 73, 81,
103–105, 107, 108, 111, 113, 115, 116,
127, 128, 130, 140
Chamaesiphon, 4, 84
Chick embryo, 26, 46, 77, 140, 188, 210
Chitin, 39, 65, 88, 89, 100, 120, 209
  media, 181
Chlamydia, 46, **140,** 210
Chlamydobacteriales, 81
Chlamydospores, 122
Chlorobacterium, 18, **53,** 207, Plate 4,
4
Chlorobium, 9, 18, **52,** 53, 158, 162,
Plate 4, Fig. 1
  chlorophyll, 9, 14, 17, 52, 158, 207
  isolation and growth of, 162
Chlorochromatium, 18, **53,** 207, Plat
Fig. 5
Chlorophyll "a," 1, 17, 53
Chloroplasts, 1, 10
Cholesterol, 7, 33, 35, 98
  test for, 194, 195
Chondrococcus, 5, 47, **135,** 210
Chondromyces, 47, **134,** 135, 210, Plate
Fig. 3
Chromatium, 43, 44, 48–51, **52,** 159, 161,
Plate 2, Fig. 6
  isolation and growth of, 159
Chromobacterium, 28, 39, 40, **86,** 209,
13, Fig. 4
Cilia, 7
Cilliobacterium, 24, **109,** 110, 210
Citrate, 5, 11, 15, 17, 31, 32, 55, 67, 75, 79
91, 93–96, 118, 119, 144
  mineral salts medium, 146
Citrate-Koser's, 156
Cladothrix, 16
Clathrochloris, 14, 52, **53,** 207, Plate 4,
3
Clonothrix, 3, 15, **82,** 208, Plate 12, Fig.
Clostridium, 5, 19, 24, 41, 71, 115, **116,**
118, 210, Plate 22, Fig. 2
Coagulase, 100
Coleomitus, 15, **128,** 129, 208, 210, Plate
Fig. 5
Colesiota, 46, **140,** 210
Colettsia, 5, 46, **140,** 210
Colloides, 12, 13, 32, **79,** 209
Conidia, 8, 20, 21, 23, 121–123, 126, 127,
Conidiophore, 23, 126
Corynebacterium, 22, 23, 24, 25, 26, 29,
111, 112, 113, 209, Plate 20, Fig. 2
Cowdria, 46, **139,** 210
Coxiella, 45, **138,** 139, 210
Crenothrix, 3, 14, **82,** 208, Plate 12, Fig.
Crista, 4, 10, 137
Cristispira, 4, 10, **136,** 137, 210, Plate
Fig. 1
Cross striation, 4, 10, 136, 137
Crystalline style sac, 10, 137
Cubical packets, 5, 6, 13, 43, 48, 101
Curved, 2, 6, 9, 16, 24, 27–30, 40, 41, 43,

, 53, 59, 64, 66, 70–73, 77, 82, 83, 88, 97, 9, 114, 116, 128, 208
drogloea, 18, **53,** 54, 207
19, 27, 45, 47, 50, 51, 57, 133, 134, 135
e, 34, 167
s, 114, Plate 21, Fig. 5
haga, 10, 19, 131, **132,** 210, Plate 27, g. 2
k-dox, 187

nia, 84
fovibrio, 41, **70,** 71, 192, 194, 208
ation and growth of, 192
an, 13, 105, 113
in, 79
ster, 42, 43, **97,** 209
ation and growth of, 192
onin test for cholesterol, 195
osphothiamine, 34, **75, 76**
lium, 189
ococcus, 14, **104,** 209, Plate 18, Fig. 4
onate, determination of, 163, 165
ion, longitudinal, 14, 16, 82, 84
ovania, 26
anospira, 10
, 2, 16, 18, 20, 40, 127, 128, 130, 133, 134, 35

ichia, 46, **139,** 210
edge tubes, 13, 105, 106, 172
entary bodies, 7
ryonic egg yolk agar, 77, 188
omycelial corpuscles, 7, 138
ospore, 2, 4, 8, 10, 15, 16, 19, 20, 24, 27, 11, 102, 115, 116, 121, 123, 127–129, 210
robacteriaceae, 87, 88, 91
ythrozoon, 47, 141, 210
pelas, 23, 113
ipeloid, 23, 113
ipelothrix, 22, 23, **113,** 209, Plate 20, Fig. 4
hrocytes, 7, 12, 26, 40, 45–47, 140, 141, 210
nia, 22, 28–32, 39, **93,** 94, 209, Plate 14, Fig. 6
erichia, 28, 30–32, **89,** 90, 92, 209, Plate 14, Fig. 2
lin, 22, 23, 112, 113
l alcohol, 7, 23, 27, 42, 48, 54, 55, 62, 63, 35, 70, 71, 94, 96, 101, 102, 106, 108, 111, 117, 161, 175
acterium, 24, 25, 26, **108,** 109, 210, Plate 19, Fig. 3
reus' carbon- and nitrogen-free medium, 190
e branching, 3, 15, 24, 25, 80, 82
hydrolysis, 41, 64, 87, 89
edia for detection of, 170
mentation of carbohydrates, 172
ribacterium, 17, **68,** 208, Plate 9, Fig. 4
ic ammonium citrate agar, 15, 17, 68, 80, 169
robacillus, 17, 19, 67, 68, **70,** 207, 208
totrophic medium for, 155
rous chloride gelatin, 169
ment, 1, 3, 7, 8, 19, 20, 23–25, 33, 35, 37, 43, 45, 52, 98, 109, 113, 121, 123, 126, 127, 130, 136, 137, 138, 141
Filter paper, 22, 29, 31, 33, 36, 41, 71, 114, 132,
  agar, 147
Fish, 12, 30, 38, 63, 64, 67, 88, 104, 113
Flagella, 1, 69, 131
  lateral, 6, 41, 73, 85, 208
  polar, 4–10, 12, 17–19, 22, 27, 28, 30–32, 38, 39–41, 43, 46, 48, 49, 51, 52–56, 58, 59–66, 69, 70, 72, 73, 82, 83, 86, 87, 102, 111, 207, 208
  peritrichous, 2, 6, 7, 15, 22, 26–32, 38, 39–41, 62, 74, 78, 84–89, 92, 95, 96, 98, 104, 109, 112–116, 127, 128, 209
  stains, 198
  subpolar, 3, 15, 80, 85
Flat sheets, 49
Flavobacterium, 32, 34–36, 39, 40, 86, **87,** 88, 209
Flexibacter, 19
Flexible cells, 4, 8, 10, 18, 19, 129, 131–133, 135, 136, 137, 210
Fluorescin, 61
Formaldehyde, 56
Formate, 6, 23, 24, 55, 56, 102, 107, 108, 109, 111, 117, 191
Fructose, 23, 24, 25, 34, 43, 55, 86, 101, 103, 106, 108, 110, 111
Fruiting bodies, 5, 8, 21, 47, 132, 133, 135, 136, 210
Fungus, nuclear stain, 201
Fusiformis, 42
Fusobacterium, 42, 43, 96, **97,** 209, Plate 16, Fig. 5

Gaffkya, 14, **101,** 209, Plate 17, Fig. 4
Gallionella, 11, 17, **83,** 208, Plate 8, Fig. 6
Gases, 13, 23, 24, 25, 31, 32, 34, 38, 41, 42, 62, 63, 64, 79, 87, 89, 90, 91, 92, 94, 96, 101, 106, 108, 111, 112, 116, 117, 209
  analysis of, 175
  apparatus for the collection and mixing of, 153
  from peptone, 6, 24, 42, 97, 98, 102, 103, 107, 108, 109, 117, 209
  preparation of, 152
  utilization of, apparatus, 151
Gas vacuole, 2, 3, 4, 9, 16, 43, 51, 52, 67, 81
Gelatin, 25, 30, 33, 34, 38, 41, 42, 56, 62, 63, 66, 67, 70, 74, 78, 86–90, 92–96, 98, 100, 102–104, 107–110, 112, 114, 115, 117, 118, 120, 123, 126, 127, 132, 169, 209
  hydrolysis of, 180
Giemsa's stain, 7, 10, 11, 26, 73, 136, 137, 141, 201, 202
Gliding organisms, 1, 4, 5, 8, 10, 15, 16, 18, 19, 129, 130–136, 199, 210
Gluconic acid, 61, 63
Glucose, 7, 22–27, 30, 32, 34, 38, 41–43, 55, 61–64, 71–79, 85, 86–90, 92–99, 103–121, 136, 209
  phosphate peptone water, 178
  tryptic broth, 23, 178
  yeast-extract tryptone phosphate broth, 100, 105, 106, 178
Glycerol, 25, 71, 110, 111, 113
Glycine, 6, 102

Glycol, 63
Grahamella, 46, **140,** 141, 210
Gram stain, 199
Gram-negative, 5, 6, 8, 11, 12, 13, 17–19, 21, 22, 27, 37, 54–66, 70–80, 82, 84–91, 93–96, 98, 100, 103, 115, 116, 127, 130–141, 207
Gram-positive, 6, 8, 12, 13, 19, 21, 27, 84, 94, 100–128, 209, 210
Granuloma inguinale, 26, 77
Granulose, 116
Green sulfur bacteria, 52
Griess-Ilosvay methods, 148

Haemobartonella, 47, **141,** 210
Haemophilus, 33, 34, 35, 37, 38, 40, **75,** 76, 188, 189, 209, Plate 16, Fig. 3
Halobacterium, 5, 12, 28, 36, 40, **66,** 67, 208, Plate 8, Fig. 4
Halophilic, 5, 28, 34, 36, 40, 42, 66, 86, 96, 101, 131, 132
  bacteria-medium for, 186
Haverhill fever, 33, 35, 99
Heidenhain's iron hematoxylin, 10, 73, 202
Hemolysis, 76, 100, 102, 103, 104, 105, 106, 108, 123
Hemorrhagic septicemia, 35, 74
Herellea, 12, 13, 36, **79,** 103, 209
Hiss's serum water, 172
Hoagland and Snyder "A-Z" trace element mixture, 143
Holdfast, 2, 3, 4, 11, 14, 15, 16, 80, 82, 84, 128, 131
Holospora, 10
Hormogonia, 3, 130
Hydrocoleus, 129
Hydrodictyon, 49, Plate 1, Fig. 4
Hydrogen, 6, 24, 36, 40, 41, 52, 58, 70, 71, 107, 147, 151, 153
  preparation of, 153
  utilization apparatus, 151
Hydrogenomonas, 19, 40, 41, **58,** 59, 147, 207, Plate 5, Fig. 6
  autotrophic medium for, 147, 150
Hydrogen sulfide, 3–10, 14, 16, 23, 25, 30, 31, 34, 41, 43, 45, 48–54, 62, 64, 67, 70, 71, 74, 87–91, 93, 94, 96–99, 104, 107, 113, 117, 129, 130, 131, 207
  medium for, 169
  production of, 167
Hyperplastic diseases, 28, 39, 85, 209
Hyphomicrobium, 8, **55,** 56, 208
Hyposulfites, 41, 70

Indole, 32, 38, 58, 62, 64, 70, 74, 76, 79, 87–90, 93–98, 109, 110, 113
  test for, 195
Iron, 3, 5, 9, 11, 14, 15, 17, 19, 56, 61, 67, 69, 70, 71, 80–84, 102, 126, 155, 187, 208
  detection of, 200
Intracellular parasites, 12

Jensenia, 22, **121,** 210

Ketogluconic acid, 61, 63
Ketone, 24, 41, 71, 108, 109, 116, 118
Klebsiella, 32, 33, 34, **91,** 93, 209, Plate 14, Fig. 4
  aerogenes group, 32, 93
Koser's citrate medium, 156
Kurthia, 22, **104,** 209, Plate 18, Fig. 3

Lactic acid, 7, 23–27, 56, 65, 71, 104, 107–1
117, 209
  dextrorotatory, 13, 22, 104–106
  extraction and determination of, 175
  inactive, 13, 22, 99, 106
  levorotatory, 13, 106
Lactobacillus, 22, 23, 24, 25, 26, **107,** 1
209, Plate 19, Fig. 2
Lactose, 7, 23, 24, 30–33, 39, 42, 61–64, 75–
85, 87–99, 101, 102, 104, 105, 108, 110–1
118, 130, 138, 209
Lamprocystis, 43, 44, 48, **50,** 207, Plate
Fig. 3
Lampropedia, **8,** 209, Plate 31, Fig. 4
Legume extract agar, 190
Leguminous plants, 27, 85
Leifson's flagella stain, 198
Leptospira, 11, **137,** 210, Plate 31, Figs
and 2
  solid medium for, 191
Leptothrix, 3, 9, 15, **80,** 83, 208, Plate
Fig. 6
Leptotrichia, 20, 26
Leuconostoc, 13, 105, **106,** 209, Plate
Fig. 1
Leucothrix, 3, 4, 15, 16, **131,** 210, Plate
Fig. 1
Levinthal media, 32, 76, 77, 188
Lineola, 26, **128,** 210, Plate 25, Fig. 2
Listeria, 22, **112,** 209, Plate 20, Fig. 3
Litmus milk, 30, 31, 36–41, 58, 62, 63, 66,
73–78, 85–88, 90, 91, 94–98, 100, 101, 1
105, 107–110, 112–114, 117–121, 123, 1
127, 174, 209
Liver extract agar, 37, 75, 188
Loeffler's inspissated serum, 31, 36, 37, 61,
77, 78, 88, 94, 97, 98, 108, 117, 123, 1
209
L-forms, 7, 20, 33, 35, 98, 138
Luminescence, 12, 31, 32, 34, 38, 39, 64,
208
Luminescent bacteria, media for, 186

Macchiavello's stain, 7, 201
MacConkey's lactose bile salt agar, 34
Macromonas, 6, **59,** 208, Plate 6, Fig. 2
Maltobionic acid, 63
Manganese, 3, 5, 9, 11, 14, 15, 17, 67, 80,
May-Grünwald, 141
Measurement of microorganisms, 197
Meat infusion agar, 12, 19, 32, 34, 35, 37,
40, 56, 57, 60, 62, 64, 65, 66, 70, 74–79,
88, 91, 93–97, 100, 101, 103–107, 111-1
170, 209
Melanin, 84
Melophagus *ovinus*, 27
Meriismopedia, 49, Plate 1, Fig. 2
Methane, 6, 13, 19, 24, 41, 42, 58, 71, 96–
101–103, 106, 116, 147, 151, 176, 209
  preparation of, 153
  utilization, apparatus, 151
Methanobacterium, 24, 41, 42, **71,** 192, 2
210
  isolation and growth of, 191
  omelianski, 24, 41, 71, **116,** 117

anococcus, 6, 13, **102,** Plate 17, Fig. 6
  mate medium for, 191
hanol, 19, 56, 58, 101, 102
anomonas, 19, **58,** 207
  totrophic medium for, 147, 151
hyl red test, 28, 30–32, 34, 79, 89–92, 178, 209
obacterium, 22, **113,** 209, Plate 20, Fig. 5
ococcus, 6, 12, 13, 14, **99,** 100, 209, Plate 17, Fig. 2
ocoleus, 129
ocyclus, 29, **72,** 208
ocysts, 8, 18, 47, 130, 132, 135, 136, 210
omonospora, 21, 23, **126,** 210, Plate 23, Fig. 5
roscilla, 19, **130,** 132, 210, Plate 26, Fig. 5
a, 12, 13, 38, **78,** 103, 209
agawanella, 46, **140,** 210
hocytosis, 22, 112, 113
axella, 36, **77,** 209, Plate 16, Fig. 2
tility tests, 197
lticellular, 1, 2, 14, 15, 16, 20, 22, 23, 81, 82, 112, 118, 120, 127–131, 210
celia sterilia, 21, 36
celium, 20, 21, 23, 24, 119, 120, 122, 123, 126, 127
cetomes, 46, 139
cobacterium, 21, 23, **118,** 119, 210, Plate 22, Fig. 3
cococcus, 12, 22, **119,** 210
conostoc, 9, 29, **73,** 208
coplana, 29, 40, **66,** 208, Plate 8, Fig. 2
  henol mineral salts medium for, 146, 155
coplasma, 7, **138,** Plate 31, Fig. 3
xobacteria, 57, 132, 183, 187
xococcus, 5, 39, 47, 132, **135,** Plate 29, Fig. 1
elomyxae, 39

amanniella, 5, 9, 17, **69,** Plate 9, Fig. 6
sseria, 13, 38, **102,** 103, 209, Plate 18, Fig. 1
sser's stain, 200
rickettsia, 46, **139,** 210
skia, 4, 17, **84,** 208, Plate 9, Fig. 2
e blue sulfate, 170, 171
rate, quantitative determination of, 148
  eduction to nitrite, 29, 31, 33, 34, 36, 40, 41, 60, 62, 63, 66, 67, 70, 75, 76, 79, 86–91, 93, 94–98, 100, 103, 104, 110, 114, 115, 118–120, 123, 149
  tilization of, 132, 136
rite
  oxidation of, 56, 57
  quantitative determination of, 148
trobacter, 19, **57,** 207, Plate 5, Fig. 5
  autotrophic medium for, 145, 148
trogen, 6, 25, 29, 31, 36, 38, 40, 60, 62, 66, 67, 85, 100, 132
  ixation, 6, 7, 27, 31, 64, 84, 85, 118, 208
  medium for, 146
rocystis, 19, 57
rosococcus, 13, **56,** Plate 5, Fig. 1
rosocystis, 19, **56,** 57, 207, Plate 5, Fig. 3
rosogloea, 19, **57,** 207, Plate 5, Fig. 4
Nitrosomonas, 19, **56,** 57, 143, 147, 207, Plate 4, Fig. 6
  autotrophic medium for, 145, 147
Nitrosospira, 9, **56,** 207, 208, Plate 5, Fig. 2
Nitrous oxide, 62, 175
Nocardia, 12, 20–22, 30, 33, 34–36, 40, **119,** 120–122, 210, Plate 22, Fig. 4
Nodules, 27, 85, 189, 209
Noguchia, 31, 39, 40, **78,** 209
Nucleus, 7, 10, 13, 27, 73, 138, 139, 140, 141
  stains for, 201

Ochrobium, 5, 17, **69,** 208, Plate 10, Fig. 1
Oscillatoria, 2, 128, 130
Oscillospira, 2, **128,** 210, Plate 25, Fig. 1
Oxidase test, 99, 195
Oxidation of carbohydrates, 172
Oxycellulose, 29, 72, 157
Oxygen, preparation of, 153

Palisades, 23, 26, 111, 114
Papain digest, 169
Paracolobactrum, 30–32, 34, 38, 85, 86, **91,** 92, 209, Plate 14, Fig. 5
Paramecium, 9, 10
Paraspirillum, 10, **73,** 208, Plate 11, Fig. 1
Pasteurella, 12, 33, 34, 35, 36, 39, **73,** 74, 167, 188, 209, Plate 15, Fig. 5
Pasteuria, 4, 16, **84,** 208, Plate 12, Fig. 4
Pear-shaped, 4, 6, 16, 84, 135
Pectate media, 185
Pectin, 62, 68, 117, 185, 209
Pediococcus, 13, 99, **105,** 106, 209, Plate 18, Fig. 6
Pelodictyon, 14, 18, **53,** 207, Plate 4, Fig. 2
Pelomyxa *palustris*, 16, 30, 32, 39
Pelonema, 3, 4, 15, **81,** 208
Peloploca, 2, 4, 9, 15, 16, **81,** 208
Peptococcus, 6, 13, **102,** 209
  medium for, 191
Peptone, 24, 25, 29, 61, 63, 97, 136
  agar, 0.1 per cent, 80, 169
  cystine sulfate medium, 169
  water, 0.5 per cent, 22, 29, 33, 35, 41, 71, 72, 106, 108, 114, 169
  yeast-extract acetate, 6, 8, 26, 43, 127, 169
  lactate medium, 71
Peptostreptococcus, 13, **106,** 107, 209
Pfeifferella, 35, 37, 39
Phenol, 29, 40, 66, 70, 113, 120
  mineral salts medium for Mycoplana, 146, 155
Photobacterium, 12, 31, 32, 34, 38, 39, **64,** 70, 186, 208, Plate 7, Fig. 4
Photosynthetic, 8, 9, 11, 17, 53, 58
Phragmidiothrix, 3, 14, **82,** 208
Pigments, 61, 85
  black, 19, 63, 84, 120, 126
  blue, 28, 112, 115
  blue-green, 1
  brown, 1, 5, 14, 29, 72, 119, 126, 134, 158
  green, 1, 9–11, 14, 18, 19, 28, 29, 44, 48, 52, 53, 72, 85, 120, 123, 126, 127, 132
  orange, 19, 67, 71, 88, 89, 100, 101, 107, 110, 118–120, 123, 126, 131–135, 158
  pink, 8, 19, 100, 126, 132, 135

  purple (violet), 4, 5, 9, 11, 14, 18, 28, 48–51, 64, 67, 86, 123, 135, 207
  red, 1, 4, 5, 9, 11, 14, 18, 28, 48–52, 63, 67, 94, 100, 101, 103, 106, 107, 110, 112, 119, 134, 135, 150, 207
  water soluble, 28, 54, 61, 70
  white, 19, 65, 100, 119, 120, 134
  yellow, 1, 11, 14, 19, 22, 23, 28, 30, 31–40, 49, 56, 58, 60, 62, 65, 67, 71, 73, 88, 89, 100, 101, 103, 107, 110, 112–114, 118–120, 123, 126, 127, 132–135, 158
Plant pathogens, 22, 28, 39, 62, 86
Pleomorphism, 5, 9, 11–13, 22, 24–26, 28, 33–35, 37, 40, 42, 43, 45, 46, 53, 54, 62, 67, 77, 85, 96–98, 102, 107, 109, 110, 112, 114, 116, 119, 120, 138, 139, 140
Pleuropneumonia, 138
Pneumococcal capsular polysaccharide, 28, 183
Podangium, 47, **134,** 210, Plate 28, Fig. 2
Polyangium, 47, **133,** 134, 210, Plate 28, Fig. 1
Polyarthritis, 33, 35, 98, 99
Polythionate, determination of, 163
Pontothrix, 15, 131
Pope and Skerman mineral salts media, 143
Potato plug medium, 88, 184
Precipitated cellulose agar, 22, 29, 31, 71, 114, 132, 156
Prodigiosin, 28, 90, 94
  extraction and identification of, 159
Propionibacterium, 12, 21, 23, 24, 25, 26, 108, 109, **110,** 209, 210, Plate 19, Fig. 5
Propionic acid, 12, 21, 24–26, 55, 102, 107–110, 112, 117, 174, 209
  identification of, 174
Protaminobacter, 28, 36, 38, **65,** 208, Plate 7, Fig. 6
  alkaline medium for, 146, 156
Proteus, 38, 45, 93, **95,** 115, 209, Plate 15, Fig. 3
Protopectinase, 92
Protozoa, 7, 9, 17, 18, 53, 73, 141
Providence group, 38, 40, 93
Pseudomonadales, 61
Pseudomonas, 19, 22, 28, 30–32, 34, 39–41, **61–63,** 85, 86, 208, Plate 6, Fig. 6
Pseudotuberculosis, 39, 74
Pseudovacuoles, 15, 43
Pteromonas, 69

Q-Fever, 138

Rabbit dung agar, 187
Ramibacterium, 24, 25, 108, **109,** 210, Plate 19, Fig. 4
Respiratory tract, 13, 14, 32, 34, 37, 42, 43, 76, 93, 97, 102–104
Resting cells, 47, 132–134
Rhabdomonas, 43, **52,** 207, Plate 2, Fig. 5
Rhizobium, 27, 32, 41, **85,** 189, 190, 209, Plate 13, Figs. 1 and 2
  medium for isolation, 189
Rhodomicrobium, 8, **55,** 161, 162, 207, 208, Plate 12, Fig. 3
  isolation and growth of, 161
Rhodopseudomonas, 43, **54,** 55, 207, Plat 3, Fig. 5
  isolation and growth of, 162
Rhodospirillum, 4, 9, **54,** 55, 207, 208, Plat 3, Fig. 4
  isolation and growth of, 160
Rhodothece, 43, **51,** 52, 207, Plate 3, Fig.
Rickettsia, 45, 95, **138,** 201, 210
Rickettsiales, 138, 139
Rickettsiella, 46, **139,** 140, 210
Ricolesia, 46, **140,** 210
Rondoletia *minor*, 64
Rosette, 4, 43, 47, 82, 131

Saccharobacterium, 28, 183
Salmonella, 34, 36, 38, 39, 89, 93, **95,** 96, 20 Plate 15, Fig. 2
Salmonidae, 34
Saprospira, 10, **136,** 210, Plate 30, Fig. 5
Sarcina, 5, 6, 13, **101,** 209, Plate 17, Fig. 5
Selenomonas, 6, 41, **73,** 208, Plate 11, Fi 2
Semisolid rabbit plasma medium, 137
Septicemia, 23, 30, 36, 113, 123
Septum, 20, 22, 29, 120, 122, 126, 132
Serratia, 28, **94,** 159, 209, Plate 15, Fig. 1
Sheath, 1, 2, 3, 4, 14, 15, 68, 80–83, 129, 13 200, 208
Shigella, 33, 34, 36, 93, **96,** 209, Plate 1 Fig. 4
  group, 33, 36, 93
Sickel-shaped, 29, 72
Siderobacter, **69,** 208, Plate 10, Fig. 2
Siderocapsa, 5, 11, 17, **67–70,** 208, Plate Fig. 3
Siderosphaera, 11, **68,** 208
Sideromonas, 17, 67, **68,** 208, Plate 9, Fi 5
Sideronema, 5, **68,** 208
Siderophacus, 17, **83,** 84, 208, Plate 9, Fi 1
Siderosphaera, 11, **69,** 208
Silica gel, 56, 57, 60, 132, 145
  preparation of, 147
Simonsiella, 2, 16, **128,** 210, Plate 25, Fi 3
Slide agglutination test, 203
Slide culture, technique for, 196
Smith-Noguchia medium for *Dialister*, 192
Sorangium, 47, **133,** 210, Plate 27, Fig. 4
Sphaerophorus, 6, 12, 42, 43, **97,** 198, 20 Plate 16, Fig. 6
Sphaerotilus, 3, 9, 15, 68, 69, 79, 80, 130, 20 Plate 11, Fig. 3
Spherical cells, 5, 6, 8, 24, 25, 27, 29, 33, 3 36, 38, 40, 44, 50, 100, 101, 102, 103, 10 106, 114
Spindle-shaped, 23, 43, 49, 52, 97, 111, 11 134
Spiral cells, 3, 4, 7, 9–11, 54, 72, 73, 122, 13 137, 209
Spirilloxanthin, 55
Spirillum, 10, 55, **72,** 73, 208, Plate 10, Fi 6
Spirochaeta, 4, 10, **136,** 137, 210, Plate 3 Fig. 3

angia, 20, 21, 83, 121, 129, 210
rocytophaga, 47, 132, **136,** 210, Plate 29, Fig. 2
k, 4, 8, 11, 16, 17, 47, 82–84, 134, 135, 208
hylococcus, 14, **100,** Plate 17, Fig. 3
ch agar, 29, 31, 71, 72, 132, 181
drolysis, 32, 33, 34, 41, 62, 64, 87–89, 97, 100, 113–117, 119, 123, 126, 132
key's medium for *Desulfovibrio*, 193
angium, 47, **133,** 210
us reaction, 35, 37
ptobacillus, 33, 35, **98,** 194, 209, Plate 17, Fig. 1
ptococcus, 14, 81, **105,** 209, 210, Plate 18, Fig. 5
ptomyces, 20, 21, 120, **121,** 122, 123, 210, Plate 23, Plate 24, Fig. 4
ptosporangium, 21, 122, **127,** 210, Plate 23, Fig. 2
ate, 29, 38, 41, 60, 70, 165
etermination of, 163
ide, 17, 48, 52, 53, 59, 81
etermination of, 163
ite, 41, 70
etermination of, 163
fur, 1–10, 14–17, 19, 29, 30, 41, 43–45, 48–50, 52, 54, 59, 70, 80, 84, 129, 130, 131, 166, 207
etermination of, 163
xtraction and crystallization, 167
fur bacteria, green, 52
ed, purple and brown, 54
nbiosis, 18, 46
nbiotes, 45, **139,** 210
angium, 47, **134,** 210

pered cells, 4, 26, 47, 97, 101, 111
rads, 13, 14, 44, 50, 99, 105, 106
trathionate, 41, 52, 60, 165
llurite agar, 113
rmoactinomyces, 21, **126,** 127, 210
obacillus, 17, 19, 29, 38, 41, **60,** 207, 208, Plate 6, Fig. 5
utotrophic medium for, 146, 155
iobacterium, 8, **59,** 84, 208, Plate 6, Fig. 1
iocapsa, 44, 48, **49,** Plate 2, Fig. 1
iocystis, 43, 44, 48, **50,** 207, Plate 2, Fig. 2
iodictyon, 45, **49,** 207, Plate 1, Fig. 3
iopedia, 43, 44, **48,** 49, 207, Plate 1, Fig. 1
ioploca, 3, 15, **129,** 210, Plate 26, Fig. 1
iopolycoccus, 45, **51,** Plate 3, Fig. 2
iorhodaceae, 48
iosarcina, 43, **48,** 207
iospira, 4, 7, 10, 30, 41, **59,** 208, Plate 6, Fig. 4
iospirillopsis, 9, **129,** 210
iospirillum, 4, 9, **51,** 207, Plate 3, Fig. 3
iosulfate, 9, 17, 29, 38, 41, 52, 55, 60, 70, 165
letermination of, 163

Thiothece, 43, 44, 48, **49,** 50, 207, Plate 1, Fig. 6
Thiothrix, 2, 3, 4, 15, **129,** 208, 210, Plate 26, Fig. 4
Thiovulum, 6, **59,** 208, Plate 6, Fig. 3
Torus, 5, 9, 17, 68, 69
Toxothrix, 14, **81,** 208, Plate 11, Figs. 4 and 5
Trellis-like, 14
Trematodes, 45, 210
Treponema, 11, **137,** 210, Plate 30, Fig. 4
Trichome, 1, 2–4, 9, 15, 16, 81, 128, 130
Trichomonas *batrachorum*, 13
Tryptose phosphate broth, 37, 75
Tubular outgrowths, 8, 56

Unicellular, 1, 2, 14, 16, 20, 48–50, 52, 53, 56, 58, 59, 61, 62, 65–67, 69, 70–90, 91, 93, 96, 97, 99, 101–119, 122, 124, 128, 130–133, 135, 136, 137
Uniseriate, 1, 9, 129, 130
Urease, 38, 86, 89, 95, 99, 100, 102, 209
  detection of, 180

Vannielia, 43, **55,** 107, Plate 3, Fig. 6
Veillonella, 13, **103,** 209
V-factor, 34, 37, 38, 75, 76, 188
Vibrio, 10, 28, 29, 30, 41, **70,** 208, Plate 10, Fig. 3
Violacein, 86
Viruses, 7, 34
Vitreoscilla, 3, 7, 16, **130,** 210, Plate 26, Fig. 3
Voges-Proskauer, 28, 30–32, 34, 79, 89, 90, 92, 95, 179, 209
Volutin, 7, 10, 129
  stain for, 200

Waksmania, 21, **123,** 210
Water-soluble pigments, 28, 54
Weil-Felix reaction, 45, 138
Wieringa's pectate gel, 185
Whooping cough, 37, 75
Wolbachia, 27, 46, **139,** 210
Wort agar, 27, 63, 186

X-factor, 33, 34, 37, 38, 75, 76, 77, 188
Xanthomonas, 22, 28, 30, 32, 38–41, **62,** 208, Plate 7, Fig. 1
Xanthophyll, 62

Yeast-extract glucose tryptone phosphate broth, 13, 63, 100, 105, 106, 178
  mannitol agar (medium "79"), 190

Ziehl-Neelsen stain, 202
Zoogloea, 38, 41, **66,** 208, Plate 8, Fig. 3
Zoogloea, 14, 17, 19, 29, 38, 41, 43, 44, 49, 50, 52, 57, 59, 68, 86
Zymomonas, 7, 27, 42, **65,** 208
  medium for, 185
Zymobacterium, 23, **111,** Plate 20, Fig. 1
  medium for, 185

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